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CONDITIONS OF GROWTH OF PHYTOPLANKTON

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PREFACE

For some time the International Council for the Exploration of the Sea has felt the necessity of the standardisation of the methods of studying the growth of plankton through joint discussion, between all those interested in the subject from different points of view, of the general problems awaiting solution. This necessity has been increasingly felt in recent years.

An important step towards this end was taken in 1928, when a special meeting of experts from several countries was convened at the University of Oslo, where, by their joint endeavours, standard methods of determining phosphates and nitrogenous compounds in sea water were elaborated (*vide* Rapports et Procès-Verbaux, Volume LIII).

The present Volume contains a series of papers which were read at a special Biological Meeting during the proceedings of the International Council at Copenhagen in 1931. The prime object of these papers is a critical review of previous ideas and knowledge about the metabolism of plankton organisms. They deal also with the results of the investigations most recently carried out, in some of which the methods elaborated in 1928 were employed. At the meeting in question a report was given of the results of work which, at the instance of the International Council, had been begun at the University of Helsingfors, with the object of standardising methods for determining the quantity and tension of carbonic acid factors in sea water.

At the conclusion of the meeting it was agreed that these general studies should be continued, and that the next step should be the elucidation of the significance of light for organisms in the sea.

JOHAN HJORT.

I. Dissolved Substances as Food of Aquatic Organisms.

By

August Krogh.

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In a pond, in a lake, in an arm of the sea and in the ocean itself we have, as on dry land, a production of organic substances from inorganic by absorption of radiant energy in plants, and a utilization of these organic substances by innumerable organisms which break down the complex compounds finally to simple inorganic substances and liberate the energy. The conditions under which assimilation and dissimilation take place in a solution differ, however, from those in air, and the processes themselves, although the same in principle, may very well show important differences. It is unavoidable that organic substances go into solution in the medium, and it is conceivable, and would even seem probable *a priori*, that the dissolved substances might be an important source of potential energy and building material for aquatic organisms including animals. The first biologist to realize this conception and to see at least its major possibilities and consequences was AUGUST PÜTTER, to whom belongs also the credit of attempting that quantitative evaluation of the conditions in natural waters by which the conception could be put to the test.

PÜTTER's main work was done in the years 1907—1909 when his book on the nutrition of aquatic animals was published. It led him to affirm emphatically the important rôle of dissolved organic substances in the nutrition of the large majority of aquatic animals. PÜTTER's methods were adversely criticized and the validity of his results called in doubt or denied by several competent investigators, and in 1913 the position of the problem was ably reviewed by LIPSCHÜTZ who came to the conclusion that PÜTTER's contentions were neither proved nor disproved. Summarizing briefly the work done up to 1913 and covered by LIPSCHÜTZ' account, I shall deal somewhat more fully with the later work.

Supply and Demand for Food in Aquatic Animals.

PÜTTER arrived at his conclusion mainly by a comparison between the nutritional requirements of a number of pelagic organisms with the supply of nutrition which they could obtain from other pelagic organisms. He tried to fortify it by a number of other considerations and experiments which will be discussed in their place, but even now a

study of the demand for food and its supply under certain conditions furnishes the strongest arguments available for his views.

The nutritional requirements of an animal can be determined quantitatively by measuring its respiratory exchange. The respiratory exchange of aquatic forms can be determined very easily by shutting them up in a closed vessel filled with water of a known oxygen content and determining the oxygen left after a suitable experimental period. The determinations are usually made by titration according to WINKLER. The exchange can be expressed in c.c. or mg. of oxygen, but for purposes of comparison it can also be stated in calories, as the caloric value of oxygen varies only slightly with the kind of material burned or catabolized.

When fats are burned or catabolized each c.c. of oxygen used liberates 4.8 cal.; for carbohydrates the value is 5.05 cal. and for proteins it is taken as 4.7 cal. The average caloric value of 1 mg. oxygen is 3.25 cal. PÜTTER gives it as 3.35 cal.

It is evident that at least so many calories must be supplied as food that the minimum respiratory exchange is covered, and if the animal is to grow or to maintain a certain amount of activity the supply must exceed the minimum demand. PÜTTER computed the minimum demand for a number of pelagic organisms, partly from his own determinations and partly from those of others, and compared it with the supply available as plankton organisms. His results were expressed as c.c. of natural water containing the quantity of plankton necessary to cover the energy exchange of his selected organisms in one hour, the volume in other words which had to be fished clean each hour. He compared this with the volume of water which would contain the same amount of nourishment in a dissolved state. In PÜTTER's first tables of this kind (1908, p. 296) the figures given were fantastic. He found for instance that the necessary plankton organisms to provide sufficient food for one hour for the sea water protozoon *Collozoum inerme* would be present in 9.4 liter of water, while the same amount could be obtained in a dissolved state from 0.55 c.c. and he rightly maintained that it would be absolutely impossible for the animal, which is 0.1 c.c. in volume, to secure each hour all the plankton algæ in 9 liter of water, while the supply of dissolved material would be ample.

In critical papers by LOHMANN, HENZE, RABEN and MOORE with collaborators it was shown that the quantity of plankton was greatly underestimated, while in his determinations of the organic substance present in solution PÜTTER had overlooked several serious sources of error which made his results many times too high.

PÜTTER admitted the criticisms of HENZE and RABEN, but maintained that, even when corrected accordingly, his figures still showed the impossibility, in the case of the majority of pelagic animals, of obtaining sufficient food from the plankton. In the case of *Collozoum* the necessary quantity of algæ could now be found in 760 c.c. and the dissolved substances in 6 c.c. of water. The ratio dropped from $\frac{9400}{0.55} = 17000$ to 127.

Again it must be admitted that if PÜTTER's figures are accepted as representing approximately the true conditions in nature his conclusion is on the whole justified, the animals would be unable to obtain more than a fraction of their food from the plankton, and no other source could be found for the main bulk than the dissolved substances.

Criticism has been raised against PÜTTER's figures for the metabolic requirements of animals by LIPSCHÜTZ who maintains that the values deduced by PÜTTER are probably too high and that the metabolic level of lower animals at a given temperature is a function not only of their size and activity, but also of the food at their disposal. This is true within certain limits for warmblooded animals and even for man himself, as shown in experiments on undernutrition by ZUNTZ and by BENEDICT and his collaborators. It holds within very wide limits for many coldblooded animals which can exist and even multiply on very different levels of nutrition. It is therefore by no means necessary to assume that aquatic animals always obtain the food necessary to maintain the metabolism observed under experimental conditions. Death from starvation is no doubt of very frequent occurrence in the sea as on land. On the other hand many aquatic animals show periods of extremely rapid growth in which their demands for food must considerably exceed the metabolism shown in respiration experiments in which no food is given.

It will be appropriate here to quote an example discussed by PÜTTER (1909, p. 69). The larva of the mosquito *Anopheles formosaensis* grows through 4 moults in 17 days from a length of 0.8 to 3.5 mm. PÜTTER calculates the increase in dry substance and, assuming that the metabolism for maintenance at each stage is equal to the amount laid on, he constructs the following table.

Growth of Anopheles larva.

Day	Length	Volume mm.	Dry substance mg.	Increase per day mg.	Necessary dry organic food mg./day	Necessary number of large Peridinids
0	0.8	0.02	0.003			
			>	0.002	0.004	200
3.5	1.2	0.067	0.010			
			>	0.005	0.010	500
7	1.7	0.19	0.028			
			>	0.019	0.038	1900
10.5	2.5	0.62	0.093			
			>	0.045	0.090	4500
17	3.5	1.68	0.25			

If the figures for the increase are not grossly in error the estimate of the necessary food is conservative, and it seems not unreasonable to ask with PÜTTER: "Is it possible that the animal can catch the requisite number of plankton organisms?"

PÜTTER's calculations were made generally invalid as proofs of his contentions by new material and information mainly brought to light after his book of 1909.

In 1908 LOHMANN published his first studies on the nannoplankton consisting of organisms too small to be caught by the plankton net, the finest of which have meshes of about 50 μ while the nannoplankton organisms are from 1 to 25 μ in diameter or length. These organisms, which include bacteria, are mainly unicellular assimilating plants

while normally only a few protozoa are present. The number of nannoplankton organisms is often extremely large and what is even more important they multiply with great rapidity, so that they will be able to supply a considerable amount of food for plankton animals without their numbers being diminished. LOHMANN showed that Appendicularia obtain their food by filtering off nannoplankton organisms. He found further that these larvæ are able to pass through their filters a volume of water per hour, many thousand times their own size. PÜTTER (1909, p. 71—83) attempts to show that even in this case the nannoplankton filtered is insufficient to cover the metabolism of the animals. He figures however, on the basis of the organisms found in the filters when these are thrown off, and apparently overlooks the fact that the animal empties the contents into its gut at frequent intervals. It must, however, be admitted that LOHMANN on his side did not prove the sufficiency of the nannoplankton food for the Appendicularia.

To determine the quantity of nannoplankton several methods are employed and a final conclusion as to their respective merits does not seem to have been reached (UTERMÖHL). LOHMANN centrifuged comparatively small samples of water and measured the volume of the organisms thrown down. In many cases this measurement is combined with determinations of the species and counts of their numbers. This method is no doubt sufficient for many purposes, but LOHMANN himself was aware that at the speeds usually employed not all the organisms were brought down. Small forms with a specific gravity near unity may easily escape, and forms containing sufficient fat to be lighter than water may be carried to the surface. It was generally assumed, however, that the errors of the centrifuge method were small only, with the exception that the large majority of bacteria escaped. ALLEN (1919) controlled the centrifuge by culture experiments. He diluted 0.5 c.c. of natural sea water which, according to the centrifuge determination, should contain 7—8 organisms to 1,500 c.c. with sterilized sea water and divided this up into 70 culture bottles so that there should be one organism (exclusive of bacteria) in about every tenth bottle. He found, however, that there were no flasks in which no growth of plankton organisms took place, while the largest number of distinctly different organisms growing in one flask was 7. While the centrifuge had shown the presence of 14,500 organisms per liter the culture experiment gave a minimum of 464,000 belonging to at least 20 species, and it was more probable that the number was over a million. Even admitting that the culture method is likely to bring out all the spores present which have no immediate value as producers of food, it must be conceded that the centrifuge method may fall very far short of indicating the available supply of particulate food for minute animals.

In 1912 a paper was published by MOORE, EDIE, WHITLEY and DAKIN which has not received the attention it deserves. These authors filtered large samples of sea water drawn up by a pump first through silk netting and thereupon through a Chamberland filter. They found near the isle of Man total quantities of organic matter not exceeding 1 mg. per liter, but their oxidation method probably gave results somewhat too low. In their discussion they say: "Tow-nettings taken with care throughout a season may show quite clearly the relative quantities of a certain organism available as food for another, but such figures taken and multiplied into volumes of water to show how many

litres of water the latter organism must filter through its body before it can make a sufficient meal from the former constitutes a drifting into ridiculous absurdity, such as usually overtakes the biologist when he applies mathematics in a blindfold fashion without thinking of the many natural limitations unrepresented in his simple formula.

A herring, which swam about aimlessly with its mouth open in a sea containing 0.5 part in one million of organic matter as plankton, would have to filter a good deal of water before it obtained an adequate supply of food, and might grow very lean in the process; another herring in the same sea making proper use of its sensory and nervous systems might be able to detect enormous variations in the distribution of food and grow fat where the other was starving".

Kolkwitz in 1911 introduced Collodion filters for the collection of the particulate material in a water sample. Such filters have a smooth surface from which the plankton collected can be quantitatively recovered. It was assumed that a separation of organisms could be obtained by using filters with different sized pores. This is not practicable, however, and the safest method is to use filters which will hold back even bacteria as described by Krogh (1930).

Nannoplankton organisms are very abundant in fresh water. Volk found in the Elbe near Hamburg up to 93,000 organisms per c.c. by the centrifuge method, and Woltereck observed a rich nannoplankton flora in the Lunzer lake, where it made up the main food of the Cladocera. It is probable from the calculations of Lantzsch that the nannoplankton in the lakes referred to by him is sufficient as food for the cladocera and similar calculations could be made on the material of Birge and Juday or of Berg and Nygaard, utilizing the chemical analyses of Berg and Krogh, but as the material must be supplemented by somewhat uncertain assumptions I think it better to desist.

In the years 1911 and 1914 the investigations by Petersen and Boysen-Jensen were published on the animal life and the organic material of the sea bottom. In the first of these papers the fact was emphasized, which had already been noted by Lohmann, that in the coastal waters of the Baltic and Kattegat a very large part of the suspended matter is not living plankton at all, but is "detritus", remains of organisms reduced mostly to unrecognizable dust, but of considerable importance as a source of nourishment to pelagic organisms and especially to those living in or at the bottom. Boysen-Jensen was able to show further that the detritus was derived only to some (probably slight) extent from the plankton organisms, but mainly from the flora of the sea bottom near the shore and especially from the grass-wrack (Zostera). This plant covers extensive areas in the shallow Danish waters down to 5—10 m. It throws off a number of dead leaves which become reduced to fragments and are partially decomposed chemically. These make up a very large part of the suspended detritus and are finally deposited all over the bottom, where they become a main source of food for the bottom fauna. Many representatives of this, especially among worms and bivalves, are regular detritus feeders. The composition of Zostera leaves does not differ essentially from that of fodder grasses and even in the detritus state the analyses and digestion experiments of Boysen-Jensen (1914) have shown it to be a food substance of considerable value. The organic detritus present in the water of the narrow seas is usually greatly in excess of organic

substances in the plankton organisms, but is very variable. Boysen-Jensen filtered 10 liter samples of water from the Limfjord near Mors through hardened filters which will hold back the major portion of the suspended matter, but will probably allow the finest particles and nannoplankton organisms to pass. He found quantities varying from 0.96 to 7.3 mg./L. Especially with the larger quantities the detritus was in great excess and organisms rare. In calm weather the detritus sinks to the bottom, but where the water is not too deep the deposition is often disturbed and large quantities again suspended. This is well shown by the experiments with detritus collectors, cylinders of 30 cm. diameter and 30 cm. depth, suspended a short distance over the bottom. Petersen and Boysen-Jensen measured the deposition in such vessels in "Thisted Bredning" and in "Nissum Bredning" in the Limfjord at depths ranging from 7 to 14 m. and found depositions varying from 0.7 g. to 52 g. per sq. m./day.

In fresh water lakes and ponds the quantities of suspended organic material — including plankton organisms — is generally larger than in the sea, but varies greatly with the seasons and also from lake to lake as shown by Birge and Juday (1922), Krogh and Berg (1931) and Krogh and Lange (1931).

It is evident from the survey given above that the general sweeping statement of the insufficiency of suspended matter including living organisms as food for the aquatic animals is untenable. Calculations cannot be based on general figures for the amount of plankton, but each single case must be studied. Animals living at or near the bottom are able to obtain large amounts of food material from the deposits. True pelagic organisms do not, as pointed out by Lohmann and emphasized by Moore, depend upon the average plankton found in vertical hauls from a certain depth, but seek out the richest layers which often contain many times more than the average.

There can be very little doubt that in the large majority of cases aquatic animals like those on land are able to obtain sufficient food in the ordinary way. They have well developed intestinal canals and many of them possess intricate structures adapted to the acquisition of particulate material. There are, however, many cases which require closer study, and there are a few where it is not unlikely that dissolved substances may play an important rôle.

Pütter (1914) lays especial stress upon the nutritional requirements of the spongiæ. He studied with great care the metabolism of *Suberites massa* and found that in the natural well nourished state it would require food corresponding to 1 per cent. of its substance per day, being able to subsist, however, on a minimum of 0.16 %. He finds that 100 g. of the sponge will filter at most 50 liter sea water per day, containing as plankton a maximum of 2.5 mg. carbon, while 30 mg. are required. It is characteristic that the natural habitat of the sponge is not even mentioned in the paper. If it lives on or near a bottom where detritus is deposited, it will probably be supplied with abundant food in the particulate state.

Pütter further emphasizes the case of *Rhizostoma* among the Coelenterata, where the gastro-vascular system communicates with the outside only through fine pores which are, according to him, wholly unsuitable to take up particulate food. According to zoological textbooks many of the openings are large enough, however, to admit sub-

stantial food particles, and there is little reason to doubt that these Coelenterates like the others catch plankton organisms.

I would mention the case of oyster larvæ studied by Gaarder and Spärck. These live on organisms of about 2 μ in size which were found in the oyster pools in numbers of 50 to 100 per cubic millimeter. One oyster larvæ will have to eat at least 1 million such organisms in the period of about one week during which its volume is doubled.

Difficulties may be present with regard to the sufficiency of particulate food in the case of animal communities, where no assimilation can take place. In the deep strata and at the bottom of fresh water lakes the amount of organic detritus appears to be abundant. Usually a surplus of organic material is deposited on the bottom and is very slowly oxidized by the bottom fauna and bacteria.

In natural caves the supply of water is, I believe, normally filtered through the soil, so that it may contain dissolved organic substances, but should be deprived of particulate material. A study of the ultimate source of nourishment for the cave fauna would probably yield interesting results.

In the depths of the sea the supply of plankton and detritus must become increasingly difficult. In all areas which can be shown to contain material from the shore there must also be a supply of organic material, and the analyses of Boysen-Jensen (1911) show that the bottom deposits contain fairly large amounts of organic carbon and nitrogen. Thus in the apparently pure sand of the North Sea bottom he found in percentage of the dry substance 0.34 % C and 0.027 % N: $\frac{C}{N} = 12.6$. In a number of localities in the Limfjord the carbon content varied from 0.58 to 4.3 % and the $\frac{C}{N}$ ratio from 10.6 to 12.4.

The clay bottom in Kattegat at depths of 30—35 m. contained 2.3 % C: $\frac{C}{N} = 11.5$ and the stinking mud of the inner part of Roskilde Fjord showed even 10.2 % C.

At great depths and far from the shore probably only the remains of plankton sink down to the bottom, and Pütter assumes that all the organic material must become dissolved before the remains reach the bottom at 3000 to 6000 meters. At depths from 2000 to 5000 m. the usual deposit is globigerina ooze, consisting largely of shells of foraminifera, but at the greatest depths even the calcareous shells disappear completely, and the bottom material is a fine red clay.

Boysen-Jensen analysed (1914) 8 bottom samples from the Ingolf expedition from depths ranging from 600 to 2,200 m. and found organic carbon in quantities from 0.3 % to 1.6 % in all of them. The $\frac{C}{N}$ ratio in the two cases examined was 10.2. It is to be assumed, therefore, that even at the greatest depths the bottom will contain some organic material which can serve as food, and such material must therefore also be present as particles in the water and be able to support some animal life. Lohmann (1909) says, that if Pütter were right in considering the dissolved organic substances as the chief source of nourishment, one should expect the depths of the ocean to be as rich in animal life as the shallow seas, while in fact they are deserts supporting only a very scanty population.

The amount of food observed in the intestine. PÜTTER maintained (1909) that in a number of cases (*Rhizostoma*, Hydroida, Alcyonaria, Rotatoriae, copepods, and several fishes) the quantities of food to be found in the intestinal canal were much too small to supply the necessary energy. In some of these cases the intestine was empty, in others only a few plankton algæ were found, and these were probably not even digested, since their green colour was retained. PÜTTER drew from these observations, which he quoted from several authors and does not appear to have ever verified himself, the conclusion that the animals in question must take up dissolved organic substances.

The observations especially of BLEGVAD (1914—1916), MARIE LEBOUR (1918—24) and also HUNT (1925) show that the conclusion is not generally warranted. A number of the forms mentioned (e. g. the copepods and several of the fishes, probably hydroids) are detritus eaters. It is true that often only a few recognizable organisms are found in their intestine, but this does not mean that it is empty. It contains an unrecognizable mass (PÜTTER himself quotes regarding the copepods discussed by him "eine grüne Masse" which is undoubtedly made up mainly of nannoplankton organisms).

In other cases, both among the fishes and among invertebrates, we have to do with carnivorous animals which take food only occasionally and the intestine of which is therefore empty when the digestion of a prey is finished. In many fishes the intestine is empty in the morning, because food is taken only during the day. In many transparent forms like larval fish the digestion is extremely rapid and the gut appears empty most of the time. For a number of the fish which, according to PÜTTER, would take only quantitatively insignificant amounts of particulate food, it can now be stated on the authority of BLEGVAD and MARIE LEBOUR, that they can and do obtain a sufficiency of such food, but the fact remains that in a number of cases the information available with regard to the quantities of food which are eaten is very incomplete.

The Dissolved Organic Substances and their Origin.

The determination of organic substances in very dilute solution is a technically difficult problem especially with regard to sea water containing 30 or more g. of inorganic constituents per liter along with a few mg. organic or even less.

The method most widely used (PÜTTER 1911, MOORE 1912, BOYSEN-JENSEN 1914, ATKINS 1922, GRAN and RUUD 1926, GAARDER and GRAN 1927) is the permanganate titration in various modifications. Permanganate boiled with organic substances in strongly acid or alkaline solution will give off oxygen to oxidize the substances present. Some compounds like oxalic acid are easily and completely oxidized in this way, while a number of others are incompletely oxidized and many not attacked at all. The usual technique is to boil a sample of the water in question with a surplus of permanganate in strongly acid or alkaline solution (in sea water alkali only can be used as acid will liberate chlorine) for a definite length of time, sufficient for the permanganate reaction to become at least approximately finished, then to add the equivalent amount of oxalic acid and titrate in acid solution with permanganate at a temperature between 50 and 60°, where practically oxalic acid only is attacked. The permanganate titration can give only mini-

mum values for the organic substances present, expressed by the amount of oxygen necessary for their oxidation. 1 c.c. of $\frac{n}{100}$ permanganate corresponds according to Pütter to 0.048 mg. oxygen. These minimum values, which vary in sea water between $^1/_2$ and 5 mg. oxygen per liter, represent an unknown fraction of the organic substance really present. Pütter designates this fraction as the "easily oxidizable" organic substances and seems to imply that these will also on the whole be more readily available for metabolic utilization than others, but this is certainly a mistake. All that can be shown by the permanganat titration of natural waters is that organic substances are present in solution in quantities which are in excess and usually greatly in excess of the quantities contained in the plankton organisms.

Moore and his collaborators measured the permanganate oxidation of sea water at room temperature and did control titrations on solution of ignited salt in distilled water supposed to be absolutely free from organic contamination. Such water is extremely difficult to obtain and probably their controls do not represent errors due to liberation of chlorine, as they assume, but organic substances present. They find no excess in pure sea water over their controls. They determine the "albuminoid ammonia" in sea water by Nesslerisation and conclude that a maximum of 1 mg. dissolved organic material per liter may be present.

Gran (Gran und Ruud 1926) and also Atkins (1922) compared the permanganate method with a biological method, keeping water samples saturated with air in closed bottles for a period of several months and studying the decrease in oxygen content, it being assumed that the organisms and especially the bacteria present would gradually use up the organic substances. The oxygen content decreased rapidly during the first weeks and then more slowly. The volume of oxygen disappearing per liter was in 3 series with unfiltered water from Oslo Fjord 2.35 and 2.81 c.c. and in the filtered samples 2.48 and 2.07 c.c. corresponding on an average to 12.4 cal. for the unfiltered and 11 cal. for the filtered samples. The permanganate determinations showed only a slight decrease in oxidizable substances corresponding during the experiment to a fall from 2.8 to 2.15 = 0.65 c.c. oxygen in the unfiltered and from 2.6 to 2.4 = 0.2 c.c. oxygen in the filtered water. This means that the bacteria must have broken down to a large extent substances which do not react with permanganate, while the total of organic substances must correspond at least to the final permanganate titration + the oxygen used up during the experiment or 23 cal. for the filtered samples. As we shall see further on (p. 17) the substances oxidized by bacteria may be only a small fraction of the total organic.

On sea water a few determinations of organic carbon have been made by oxidation with potassium bichromate and sulphuric acid. As shown by Raben (1910) Pütter's very high results are due to errors in the analytical procedure. Henze (1908), who determined the organic carbon as CO_2 along with the preformed CO_2, found differences which were too small to be analytically significant, and Pütter's attempt (1909, p. 100) to construct them into an argument for the presence of 4 to 5 mg. organic carbon pro liter is unwarranted. Raben himself found in sea water from the Baltic 3—3.2 mg. C. pro liter, while samples from the Kiel Fjord gave values between 11 and 14 mg. Calculated

as calories on the assumption that the substances were carbohydrates, we find 30 cal. in the Baltic and 110—140 cal. in Kiel Fjord. It will be noted that RABEN's result for the Baltic does not differ significantly from GRAN and RUUD's value for the Oslo Fjord, but it must be admitted, I think, that it is at present impossible to give any really reliable figure for the organic matter dissolved in sea water. Experiments to settle this problem are in preparation in the laboratory of the writer.

On fresh water much more comprehensive and reliable determinations are available. BIRGE and JUDAY with their collaborators studied a number of samples from lake Mendota and other North American lakes and rivers. They removed the plankton and detritus from very large samples of water, 150 to 500 liters, by centrifuging, evaporated down the water to dryness in vacuum pans and examined the residues by chemical analysis according to recognized methods. They found as an average for lake Mendota 5.80 mg. carbon and 0.393 mg. nitrogen per liter giving the ratio $\frac{C}{N} = 14.8$. The single samples varied from 4.00 to 7.95 mg. C. and 0.304 to 0.559 mg. N. Similar results were found in other lakes; lake Michigan giving the exceptionally low values of 3.24 C. and 0.143 N. In the Wisconsin river the very large quantity of 15.23 mg. C. was observed. The amount of ether extract is about 0.5 mg./L. Correcting for this the rest of carbon and nitrogen can be computed as "crude protein" and carbohydrate by making the usual assumption that the nitrogen found is 16 % of the protein and that this contains 53 % C.

BIRGE and JUDAY state that the carbohydrate present contains only a negligible amount of sugars. The average results for lake Mendota are per liter 0.56 mg. fat, 9.27 mg. carbohydrate and 2.76 mg. crude protein. The corresponding averages for the plankton contained in 1 liter is 0.15 mg. fat, 0.95 mg. carbohydrate and 0.88 mg. protein. It will be seen that the quantities present in solution are much larger than those in the plankton. Calculated as calories we find in solution 56 cal. as against 7.7 in the plankton.

On a number of samples the nitrogenous substances calculated as "crude protein" were subjected to further analysis by DOMOGALLA, JUDAY and PETERSON (1925). About $^1/_4$ of the nitrogen, 0.1 mg. or 100 γ (microgram) per liter is present as genuine protein which can be precipitated with mercuric chloride and other reagents. The rest is present partly as amino acids (78 γ nitrogen), partly as peptides (150 γ), but other nitrogen compounds are also found.

PETERSEN, FRED and DOMOGALLA have further isolated and determined a number of of amino acids, giving characteristic reactions, and found for instance per liter from the surface of lake Mendota 10 γ tryptophane-N, 10 γ tyrosin-N, 6 γ histidine-N, 1.5 γ cystine-N, 40 γ arginine-N and 8 γ purin-N.

It cannot therefore be doubted that the water contains a number of substances essential as building material in the animal body, but it must be remembered on the other hand that most of these are present in the very high dilution of 1 to a hundred million or less and that the non nitrogenous substances do not appear to be in forms which can easily be absorbed or utilized by animals.

KROGH and LANGE (1931) utilized the combustion methods worked out by KROGH for a study of the organic substances present in the lake Furesö and their variation during

the year. It was found possible (Krogh 1930) to burn organic substances in quantities of a few mg. by means of copper oxide and to determine by gasanalysis the CO_2 formed, the nitrogen liberated and the oxygen used to reoxidize the copper. All suspended material, including all organisms even to the smallest bacteria, is removed from the water by filtration through special collodion filters, and this material is subjected to analysis. The purified water is evaporated down to a small bulk, certain salts which interfere with the combustion are removed, substances present in colloidal solution are filtered off, and the concentrated sample finally evaporated down to dryness in a combustion tube. Special determinations are also made on the material present as colloids.

On the average we find in the surface of the Furesö in mg. pro liter:

	Plankton	Colloid	True solution
Nitrogen	0.09 ± 0.045	0.06 ± 0.044	0.52 ± 0.092
Calories	6.8 ± 3.1	4.1 ± 1.35	46.8 ± 2.1

As in the American lakes the organic substances in solution are greatly in excess of those present in the plankton. The latter show considerable variation according to the seasons, but the matter in true solution remains remarkably constant throughout the year and cannot be involved to any considerable extent as food for the organisms.

To study this problem of the availability an experiment was made somewhat similar to that described by Gran and Ruud. Samples of water from the Furesö were kept in darkness for nearly 4 months. From a paperfiltered sample 2.8 c.c. O_2 disappeared pro liter and from a sample sterilized by filtration through collodion 2.1 c.c.

The combustion analysis made after 4 months showed a loss of only 1 calorie dissolved substance for the paperfiltered and 2 for the sterile water. It is to be assumed that the colloids have been oxidized, and the experiment shows that the substances in true solution have been in the main unsuitable as food for the organisms present. I do not expect that similar results will be always obtained. It seems very probable that microorganisms will be occasionally present which will break down the organic matter of lake water.

In ponds the amount of organic material in solution may be much larger. Krogh (1930) gives analyses from a small pond containing about 160 cal. per liter. About half of this was present in the colloidal state, but a sharp separation could not be effected.

The origin of the dissolved organic material. Pütter postulated (1909, p. 112) that the plankton algæ give off to the water the large amount of organic material found. His calculations showed that the quantities derived from rivers, from the bottom flora and from the land generally must be insignificant for the ocean taken in its entirety and even for such waters as the Baltic or the Mediterranean. Since, according to his theory, large amounts were used up by animals, it became a necessary consequence that they should be produced and liberated by plankton algæ.

As evidence for the production Pütter (1908) gives a series of 17 experiments in which samples of sea water were placed in light (natural daylight, Naples, February) and darkness. Two samples were unfiltered, while two were filtered through filter paper which was supposed to hold back all the algæ and to let through all the bacteria. In

about half the unfiltered samples oxygen was liberated in light, in the other half oxygen was absorbed. There is a slight average excess of liberation. In all the filtered samples in light oxygen was absorbed, but in darkness oxygen was found to be liberated in two cases from the unfiltered and in two cases (in small amounts) from the filtered water.

Pütter believes that the algæ must have produced organic substances corresponding to the difference between the oxygen change in the filtered and unfiltered samples in "light" and calculates in this way an assimiliation corresponding per liter to not less than 0.99 mg. O_2 in 24 hours or amounting to 0.37 mg. carbon. Pütter calculates that this is at least 10 times the quantity which can have been present in the algæ, and almost the whole must therefore have been liberated, probably as sugar, into the water! The theoretical basis for this calculation is extremely doubtful, and the experimental results themselves, showing oxygen production in darkness even in cases where the same water shows oxygen absorption in light, cannot inspire confidence. Pütter calculated that on an average his algæ liberated 0.18 mg. O_2 per liter in 24 hours in complete darkness.

In a series of experiments published in 1911 Pütter attempted to demonstrate the liberation of organic substances directly by permanganate titration.

A suspension of freshly collected plankton consisting almost exclusively of diatoms was added to 10 liter sea water. Pütter makes the remarkable statement that the quantity added was never large enough to influence the titration value of filtered and unfiltered water. Determining the free oxygen and permanganate oxidizable substances (expressed as O_2) at intervals he finds that both diminish at first and during the night, but increase during the following day. This is taken to prove that the oxidizable substances formed through assimilation were given off to the water. It is to be hoped that for these determinations the water was filtered! There is no proportionality whatever between the increase in free O_2 and the corresponding increase in permanganate O_2 and the 4 experiments made do not allow any valid conclusion.

Experiments of a similar kind to those made at Naples were undertaken by Pütter in Kiel Harbour (1924). The results again show a calculated assimilation far in excess of what can, according to Pütter, be stored in the algæ. From the determinations of the oxygen used up by the water in darkness Pütter calculates the number of bacteria per c.c. as varying between 100,000 and 540,000 and to be on an average 300,000, while the bacteriological tests (Kisskalt) failed to reveal more than to a maximum of 42,000 while the minimum was 11! The figures for the oxygen liberated by algæ show the most amazing variations. In experiment 12 for instance the oxygen production of the first 24 hour period is given as 10.46 mg.! while during the next it is only 0.83 mg. The whole series varies from 0.12 to 10.46. To average such figures without paying any attention to the causes of variation is unwarranted.

Pütter assumes for the algæ present in 1 liter a fixed volume of 0.5 cmm., corresponding to not more than 0.1 mg. oxygen combustion value, while the "average" 24 hours production amounts to 2.4 mg. It does not seem to have occurred to him that the algæ may show a vigorous growth in his experimental bottles.

In culture experiments made by Gaarder and Gran the species and number of

algæ present in the water were determined before and after the experiment in which the liberation of oxygen was measured. In one 3-days experiment the oxygen liberated amounted to 0.86 mg., and it is calculated that this must correspond to the combination of 0.32 or at least 0.2 mg. carbon. The algæ present increased in number from about 30,000 to about 200,000 corresponding to an estimated increase in weight of 7 mg. Assuming a carbon content of only 1 %, this works out as 0.07 mg. carbon, and it is concluded that between 0.2 and 0.3 mg. or 3—5 times the amount stored in the organisms must have been given off to the water "in a soluble or colloid-disperse form". This estimate is very low compared with PÜTTER's, but I think it must be admitted that even in this case the estimates of the carbon combined in the protoplasm of the organisms are too crude to allow any safe conclusion.

KROGH and LANGE (1930) made the following experiments. Samples of artificial solutions of suitable salts were inoculated with a pure culture of the alga *Scenedesmus obliquus* and incubated at about 25° in closed bottles exposed to strong artificial light for periods from 3 to 6 weeks. A rich growth took place, and at the end of the period the algæ were collected, dried and analysed. The culture solution was evaporated and likewise analysed by combustion. The analyses showed organic material corresponding to 374 cal. in the algæ and an increase corresponding to 16 cal. in the water. Of the organic material formed by assimilation 4.4 % was dissolved and 95.6 % was found in the bodies of the algæ.

Two paperfiltered and unfiltered samples of natural water from the lake Furesö were incubated in strong light for 6 weeks at 23° after addition of small amounts of ammonium phosphate. Both in the filtered and the unfiltered water a rich growth of algæ (*Nitzschia*, *Chlorella*, *Scenedesmus* and other forms) took place, and oxygen was liberated to the extent of over 20 c.c. The dissolved organic substances in the water became increased, but the increase was less than 10 % of the increase within the organisms.

In a final experiment natural water was again employed, but was filtered through collodion holding back all organisms and thereupon inoculated with plankton material excluding crustaceans. The algæ were allowed to grow for 18 days when their dry weight had increased from less than 0.1 to about 50 mg. corresponding to about 210 cal., while in the water there was an increase corresponding to 21 cal. or just 10 %.

In so far as these results can be generalized[1]) they show that the organic material produced by algæ is only to a slight extent given off to the water, while 90—95 % are stored in the organisms. This means that PÜTTER's theory cannot be generally valid. The assimilation produces practically all the organic material on which the animal organsisms must feed and to obtain it, they have to eat the plants. On the other hand the result does not exclude the two possibilities that aquatic animals derive a certain small

[1]) It might be argued against these experiments that the conditions for growth were optimal. It is conceivable that when algæ assimilate without being able to grow (in the absence of nitrates or phosphates) the carbohydrates may be given off to the water. Preliminary experiments to test this possibility show that *Scenedesmus* can even under these conditions store a considerable amount of carbohydrate, the dry substance of a sample increasing in a 4-days experiment from 11.9 to 17.6 mg. During the same period about 6 mg. carbohydrate was given off to the water.

fraction of their nutritive material directly from the water or that certain forms get their food mainly in this way.

The evidence points to the dissolved substances as being mainly remains of dead organisms and waste products. The 5 to 10 % of the production found in the experiments just cited may have been given off by the growing algæ, but they may just as well have come from dead algæ in the pure culture experiment and further from bacteria and animal organisms (protozoa) in the natural water experiments. Experiments by KROGH (1930) on the absorption by animals of organic substances, which will be discussed later, show that mussels, daphnia, fishes and tadpoles even in a state of inanition give off organic combustible substances to the water in quantities amounting to 10 % or more of their metabolism. When plankton organisms die off (for instance at the end of a maximum) their bodies will largely become dissolved as colloids and perhaps later be decomposed sufficiently to go into true solution. There can be no doubt that many of these dissolved substances can be readily utilized by microorganisms — especially bacteria, but the experiment referred to above (p. 17) appears to show that the permanent stock of dissolved combustible substances in the Furesö is not readily accessible even to bacteria. The concentration must therefore become fairly high before an equilibrium between production and utilization can become established. This result is corroborated for sea water by an experiment to be mentioned later (p. 25).

The Experimental Evidence Concerning the Ability of Aquatic Animals to Utilize Dissolved Organic Substances.

PÜTTER has made a large number of experiments to test his theory on fishes, actinia, ascidia and copepods, and he finds in them all clear evidence, amounting in his opinion to proof, that a large proportion of the metabolism is covered by the absorption of dissolved substances. It will be convenient first to examine this evidence together with the experiments on fishes made in PÜTTER's laboratory by LIPSCHÜTZ and next to proceed to other contributions to the subject.

PÜTTER's experiments on fishes. The composition of the body of a fish can be determined by analysis and the heat of combustion or "oxygen capacity" calculated. When a number of fishes of the same species and approximately the same size are analysed, the heat of combustion of a similar animal can be inferred. If now such a fish is kept in an aquarium without particulate food for a suitable length of time, during which the respiratory exchange is determined, the loss in combustion value can be determined from the respiratory exchange and compared with the final combustion value found by analysis. If the two values agree the fish lived during the inanition period on the substances of its own body. If the loss calculated from the respiration experiment is in excess of that found by analysis the fish must have taken up substances from the water corresponding to the difference. This reasoning of PÜTTER's is of course perfectly sound, but to be conclusive the analyses and respiration experiments must be carried out with a great deal of care.

PÜTTER made his first experiments on goldfish and sticklebacks and found that

when kept in pure water (Göttingen waterworks) the loss in combustion value calculated from the analyses and from the respiration experiments showed agreement within 5 %. When nutritive substances (asparagin and glycerin) were added the fishes lived longer and the loss calculated from the respiration experiments was much larger than that found by analysis. In one experiment on 3 sticklebacks Pütter even calculated an increase in protein built up from the absorbed asparagin! As pointed out by Lipschütz (1913) only single animals were analysed, and it would seem that Pütter himself does not claim these experiments as convincing, since they were not mentioned in his book.

A series of experiments were made by Pütter on 5 species of sea water fishes kept in the aquaria at Naples. By analyses of animals thought to be representative the combustion value was determined. The experimental animals lived without food in water which was constantly renewed, and each day a respiration experiment, usually on a single animal and of ½ to 1 hour's duration, was made. At the end of the experimental period again an animal was analysed. In all cases the oxygen used according to the respiration experiments was far in excess of the combustion value of the tissues catabolized according to the analyses. The excess must have been utilized for the combustion of absorbed material and this amounted to about 50 % or in some cases even 75 % of the total catabolized. A calculation, based on a preserved specimen of Gobius, presented to Pütter and stated to have lived in an aquarium without food for 417 days, and giving 88 % of the substances catabolized as absorbed from the sea water, is scarcely to be taken seriously.

The most complete series of experiments was made on Heliastes. One series of grown animals with analysis of one single fish as control before the experiments of 48 days duration showed an absorption of 64.5 % of the total metabolism. Another on half grown animals of 20 days duration showed only 26.5 % absorption.

In a further experiment on grown fish hydrolysate of Heliastes was added to the water in quantities amounting to 1 mg. N. pro liter. The water was changed every day. The calculated absorption in a 14-days experiment was 56.6 % of the total metabolism. In a fourth experiment hydrolysate of the alga *Ulva* was added in quantities corresponding to 0.145 mg. N. pro liter. In this 10-days experiment practically the whole of the metabolism could be computed as covered by absorption of dissolved substances. It is characteristic that the 3 analyses used to calculate the initial composition of the fish show differences which are about as large as those produced by the inanition.

	Exp.	Percent of fresh weight			Percent of organic				
		Water	Ash	Organic	Protein	Fats	Carbo-hydrate	Extract	Insoluble N-free
Initial	1	72.0	7.9	20.1	67.8	9.4	2.3	10.9	9.6
	2	74.4	7.1	18.5	58.0	4.8	5.5	19.5	12.2
	3	75.4	5.6	19.0	51.9	8.0	6.7	10.6	22.8
After starvation	1	75.4	7.1	17.5	66.0	2.1	1.9	14.0	16.0
	2	70.2	7.4	15.4	64.3	2.4	9.0	13.1	11.2
	3	74.9	6.3	18.8	63.2	6.2	2.2	16.4	12.0

It is evident, when the initial values differ so much, that the determination of the absorbed quantity of dissolved material cannot be very accurate, but even admitting this the experiments seem to show that a large proportion of the observed metabolism cannot be accounted for by the observed loss of body substance. This conclusion is, however, made invalid by the experiments of Lipschütz.

Lipschütz, working in Pütter's laboratory, repeated and extended the experiments on the utilization of dissolved substances, but his results are very different, and serious sources of error, which had escaped the notice of Pütter, were detected by him. Pütter had assumed that the amount of dry substance in a fish could be calculated with sufficient accuracy from the length when a few individuals of the same length had been analysed. Lipschütz gives a number of determinations on small carp showing that this assumption does not hold. Lipschütz detected a source of error in the respiration experiments which were of too short duration. When a fish is caught in the aquarium and transferred to the experimental bottle, it becomes restless and during the first hours its respiratory exchange may be increased. Comparing experiments of 1—2 hours duration with such of 4—6 hours on the same fish the oxygen absorbed per hour was always less in the longer experiment and the difference varied from 1 to 60 %. The difference between the exchange in the later hours, when the fish has quieted down, and in the first half to one hours is of course larger still.

In his own experiments Lipschütz reduced the first source of error by analysing a larger number of animals and the second by always comparing the exchange in the experimental solution with that observed in a control experiment in pure water. He worked first on small carp in asparagin (20 mg./L.), glucosamin (20 mg.) asparagin 20 mg. + glucose 20 mg. and finally tyrosin 17 mg. + glucose 20 mg. The two first series showed that the substances added had not been utilized. The two last showed differences from the controls indicating a utilization of the added substances to the extent of 13 % of the total metabolism in asparagin-sugar and 32.7 % tyrosin-sugar, while at the same time the control series in pure water showed a larger loss according to the analyses than according to the respiration experiments. The figures are as follows:

	Calculated weight[1]) g		Combustion value mg O			%	O_2 absorb.	Exchange from dissolved	
	Before	After	Before	After	Diff.	Diff.	mg.	mg.	%
Pure water	4.448	3.349	3643	2488	1155	31.8	971	—184	—19
A + sugar.....	6.843	5.402	5604	3973	1631	29.1	1876	245	13
T + sugar.....	6.599	5.461	5402	4015	1387	25.7	2062	675	32.7

There is some difference in the percentage loss of tissue substances, but scarcely enough to be significant, and the large difference in the utilization of "dissolved substances" is mainly due to the large respiratory exchange in the two last series. This high exchange was observed in pure water before the "feeding" was begun, and Lipschütz concludes therefore that the differences are due to accidental differences in the nutritional level of the 3 batches of animals, the first batch being more or less starved beforehand in

comparison with the two others. A determination of the weight of the animals compared with the calculated weights would have revealed this[1]).

The conclusion of LIPSCHÜTZ is borne out by a study of the respiratory exchange of the 3 groups. The first remains at a low and nearly constant level, while the others show a gradual decrease during the experimental period of 25 days. This decrease is especially large for the third group and they all reach about the same level. I do not think it warranted to conclude with LIPSCHÜTZ that these experiments prove that no utilization of dissolved substances has taken place, but they do show that such a utilization can only be rather slight.

Similar experimental series were undertaken on elvers of which one group was kept in pure water, while a second was "fed" with asparagin in sugar and a third with extract of daphnia. These series failed to demonstrate any absorption of the dissolved substances.

In 1911 PÜTTER published two papers on the metabolism of ascidia and actinia respectively. In both he kept the animals in closed receptacles with sea water for a period of about 24 hours and measured the oxygen absorption. He determined further the volume of $\frac{n}{100}$ permanganate which could be decolorized by 100 c.c. of the water before and after the experiment. With the ascidia he made 4 parallel determinations of the same duration and in vessels of the same size on two groups of animals of which I give one set as an example.

No.	Tp.	Number	Weight g.	Duration hours	O_2 absorb. mg./L.	Permanganate mg. O/L. Before	Permanganate mg. O/L. After	Increase
3	10.6	4	237	21.8	5.15	1.22	2.12	+0.90
4	10.6	3	134	22.0	3.37	1.22	1.53	+0.31

He assumed 1) that the animals gave off oxidizable substances and absorbed others, 2) that the quantity given off is proportional to the oxygen absorption and 3) that in both experiments the same amount of nutritive substances — the total quantity available — was absorbed. None of these assumptions is supported by facts and there are no control experiments in water without animals. The second assumption differs significantly from the assumption made with regard to actinia namely that the excretion for the same group of animals remains constant in spite of large variations in oxygen absorption. Putting the excretion down as x and the absorption as y he proceeded to solve the two equations

$$5.15\,x - y = 9.90 \text{ and } 3.37\,x - y = 0.31$$

from which $x = 0.328$ and y the absorption $= 0.795$. There is no attempt to estimate the effect of errors in the permanganate titration which at its best may give variations amounting to 0.1 mg. oxygen per liter. In many cases a sharp endpoint cannot be ob-

[1]) For some reason the animals were not weighed, but their weight was calculated — as in PÜTTER's experiments — from their measured length by comparison with weighed specimens under the assumption that the weight is proportional to the cube of the length. This assumption is inaccurate and may lead to grave errors as shown by KEYS.

tained at all, but the colour will fade at first in a few seconds and then gradually more slowly. In the study of the lake Furesö by KROGH and LANGE it was intended to compare the permanganate titration with the combustion determinations of the dissolved organic substances, but the attempt was given up because of the uncertainty of the permanganate titrations. When permanganate titrations showing rather small differences are used in equations like PÜTTER's the results may become monstrous. PÜTTER had the misfortune to obtain fairly consistent values (from 0.46 to 0.80) for the absorption in the 4 pairs of determinations. The excretion, however, which is assumed to be constant per mg. oxygen in each pair of determinations, shows very wide variations (between 2.0 and 12.9 per kg. animal in 24 hours) according to my calculations.

In the experiments on actinia, made at the same time, the same group of animals is used in a series of experiments in which the content of permanganate oxidizable substances in the water varied from 0.57 to 1.39 mg. O/L. In some cases the quantity increased during the 24 hour experimental period, while in others it decreased, but PÜTTER found that an increase took place in the 4 experiments with a low content (0.57—0.96) and a decrease in 5 with a high (1.10—1.34). Assuming a constant value for the supposed excretion of oxidizable substances (cf. above) he proceeds to calculate the absorption, but in this case again the arbitrary assumptions and the errors inherent in the methods make the results unreliable to the extent that they cannot even be accepted as evidence for the existence of absorption of dissolved substances.

It will be convenient to mention here one further series of experiments by PÜTTER (1925) on the exchange of marine copepods in natural water and after the addition of 10 mg. glucose per liter. The experimental procedure is similar to that employed in the ascidia and actinia experiments in so far as the metabolism is calculated from parallel experiments by solution of equations with two unknowns.

The technique, which is in the main described in another paper (1923), appears to be as follows. Two vessels of one or two liters contain water from Kiel harbour with the copepods caught with a small net. To one bottle glucose is added corresponding to 10 mg./L. The water in the vessels is stirred and after some minutes 3 experimental bottles are filled through a syphon from each vessel. One of these is used at once for the determination of oxygen. In filling the two others care is taken to get a different number of copepods into each. After an experimental period of 1 to 3 days the oxygen in the bottles is titrated and the copepods distributed in classes of small, medium and large size and counted, the numbers found being recalculated as corresponding to a certain number of normal copepods called Oithona. It is assumed 1) that the samples taken at first for O_2 determination represent exactly the initial oxygen content in the experimental bottles, 2) that the water contains everywhere the same number of "bacteria" — that is organisms other than copepods, having a definite gas exchange, 3) that the calculated numbers of Oithona are proportional to the gas exchange of the copepods actually present in the experiment and finally tacitly 4) that reproduction of bacteria and copepods takes place at the same rate in the bottles which are to be compared. On these assumptions the metabolism of both "bacteria" and copepods is calculated from each pair of experiments. The calculated metabolism of the copepods is only about 10 % or less of the

"bacterial", which fact alone make the results very problematic on account of the accidental errors, even if the assumptions above enumerated were absolutely justified. It is not surprising, and PÜTTER himself does not seem to be surprised, that the metabolism calculated for 100,000 Oithona-hours turns out to be 0.36 mg. at 22°, 0.75 at 16.5° and 0.87 at 8.5°, while it ought to be about thrice as high at 22° as at 8.5°. PÜTTER strikes an average, but forgets to mention a temperature at which this average should be valid. For the glucose experiments the values calculated are 1.56 mg. at 22°, 3.16 at 16.5° and 3.83 at 8.5° which does not show anything about the direct influence of sugar upon the metabolism of copepods, the more so as the food organisms of the animals may very well have multiplied. The highest figures are found at the lowest temperature, where the experiment lasted 3 days. It is evident that the technique employed is unsuited to the purposes of the experiments. PÜTTER mentions that he had at his disposal filters by which he could remove all solid particles including bacteria. If he had studied the metabolism of copepods in filtered water, he might have had a fair chance of solving his problem.

What is shown by PÜTTER's experiments is the very large increase of about 600 % in total metabolism of the samples, caused by the addition of 10 mg. sugar. This increase must be mainly, and is probably wholly, due to the "bacteria". The water contained originally about 12 mg. carbon, dissolved in organic combination, pro liter. To this the 10 mg. sugar adds 4 mg. When an increase from 12 to 16 mg. can increase the metabolism 6-fold, it can only mean that the organic compounds originally present must be of very poor quality in comparison with the sugar and PÜTTER's experiments, rightly interpreted, therefore corroborate the conclusion from the experiments of KROGH and LANGE on fresh water that the organic substances in solution in natural water are mainly waste products.

Especially in his later work PÜTTER made extensive use of statistical methods and he undoubtedly deserves credit for introducing these methods to many biologists, although it is a mistake to believe that he was the first to utilize them in biology outside genetics. He seems, however, to have overlooked the fact that by no amount of mathematics or statistics can valid conclusions be extracted from unreliable experimental material.

The experiments of KNÖRRICH, WOLFF and KERB. KNÖRRICH (1901) was the first to maintain, several years before PÜTTER, that aquatic animals could live on dissolved substances. He kept daphnia in a filtered and sterilized infusion of straw which was renewed every day and found that they would live for a fortnight and produce a small number of offspring which grew up in the infusion. It was shown, however, by WOLFF (1910) that an infusion prepared according to KNÖRRICH contained very fine organic particles which could be thrown down in the centrifuge and this material was found suitable as food for daphnia.

WOLFF himself found that females of the daphnia Simocephalus would live for more than 21 days in aquarium water filtered through Chamberland filters, but would die in 10 days in tap water. They would pass through several moults and produce eggs and young which would moult every 3 days, while in tap water the young would moult once only and live for 12 days. WOLFF took this to prove that the animals must take

up dissolved substances from the aquarium water which was supposed to contain them. It is, however, just as possible that the combination of salts in old aquarium water was more suitable to the animals than that of fresh tap water. KERB (1910) and also BIEDERMANN (1911) point out that the apparent growth and moulting does not prove that the amount of dry substance increased or was even maintained, and KERB tested this objection experimentally by repeating WOLFF'S experiment, combining it with weighing of dried samples of the daphnias. The three samples at the beginning of the experiment weighed respectively 105, 110 and 111 mg., after 4 days the same number of animals weighed 55, 60 and 66 mg. and after a week more 24, 27 and 28 mg. Numerous moults took place and there is no reason to doubt that the animals were simply starving. The decrease in weight could not be prevented by the addition of peptone (100 mg./L.) to the water.

In other experiments KERB added 50 mg. glucose to 100 c.c. of water in which 12 larvæ of the mosquito *Corethra* were kept for 11 days at very low temperature ($^1/_2$—3°) and found no significant decrease in the quantity. It is very doubtful if the metabolism of animals with a total weight of only about 100 mg. at the low experimental temperature can give valid results.

The experiments of OEHLER and PETERS on protozoa. It is well known that bacteria can take up and utilize organic substances from very dilute solutions and the same is no doubt true for many unicellular forms and even for higher algæ. LOHMANN pointed out that among the animals an absorption was most likely to be found among protozoa, because their surface per unit weight is extremely large. It is, however, an open question whether such absorption does really take place in protozoans generally.

OEHLER succeeded (1917) in rearing pure cultures of different amoebæ and later (1919) of two flagellates, *Bodo* and *Prowazekia*, and one ciliate, *Colpoda steinii*, which would thrive and multiply indefinitely on dead bacteria of one single species. OEHLER tested these forms on various culture media without particulate food on which they invariable succumbed. These media were: 1 % peptone + 1 % glucose, bouillon agar, several proteins, albumoses, meat extract, amino acids with and without sugar. When the protozoa were transferred to such media, they would exist as dwarfs for a short time on the bacteria transferred with then, and then die out. They would thrive, however, on these same media when fed on dead bacteria of different kinds or small yeast cells. They would thrive equally well on artificial fine emulsions of boiled fish muscle or single proteins like casein and edestin. These forms therefore appear to be adapted to particulate food which is brought into solution in vacuoles inside their protoplasm. OEHLER is of opinion that they are able to take in drops of the fluid media in like manner, but suggests that the concentration of the small quantities which they can obtain in this way may be insufficient for their needs. He points out, however, that certain flagellates like *Polytoma* can be cultivated indefinitely in sterile solutions.

PETERS cultivated the common hay infusorian *Colpidium colpoda*, which normally feeds on bacteria, in a sterile medium containing in mg./L. glucose 300, histidine, arginine and leucine 100 of each and ammonium lactate 30, a total of 630 mg. organic corresponding to about 2500 cal. The animals multiplied up to 8,000—10,000 per c.c. on about the 12th

day after inoculation when a diminution in living material began to take place with a decrease both in number and in size of the individuals. I calculate the maximum as about 70 mg. fresh weight or probably less than 10 mg. dry weight per liter. There is good reason to believe with PETERS that the animals will use up in the course of a 40 days experiment practically all the available energy, and it must be inferred therefore that they can take up the organic substances not only from a fairly concentrated solution, but also when it becomes diluted down to the concentration in most natural waters[1]).

The *colpidia* are able to utilize a variety of organic substances including fatty acids with more than 3 carbon atoms glycerine, cane sugar and glucose + lactate, but neither glucose nor lactic acid when alone available, while ammonia seems to suffice as a nitrogen source. This protozoan, which is probably representative of many others, can evidently thrive and multiply on solutions and can apparently exist even on very dilute solutions of organic substances although in this case it becomes dwarfed.

The experiments of KŘÍŽENECKÝ and others. A series of papers were published between 1926 and 1928 by KŘÍŽENECKÝ and his collaborators dealing mainly with the rearing of tadpoles in solutions containing organic substances. The substances used were peptone + sugar and "bioklein" a preparation from the germs of cereals containing about 20 % proteins and their split products and about 60 per cent. sugar and dextrin. In most experiments the substances were employed in concentrations of 1 to 4 g. per liter, and it was found that tadpoles kept in such solutions, which were changed every day, would grow and show considerable increases in dry substance, while the tadpoles in pure water might show a slight increase in length, but always a large decrease in live weight and dry substance. The solutions could not, however, produce the rate of growth shown by control animals regularly fed on particulate food, though in a few cases the bioklein animals did not fall far short of this. It is a remarkable feature that the results in the different series with the same or nearly the same amount of the same kind of dissolved nutrition differ greatly, the growth being often slight, but sometimes nearly normal. Influences must exist which have escaped the notice of the experimenters.

In similar experiments on the larvæ of *Amblystoma* and *Triton* F. BOCK failed to find any significant difference between the animals kept in pure water and those in peptone + sugar or bioklein in concentration 1 g./L. The corresponding experiments on frog tadpoles showed only slight, but probably significant, differences between the

[1]) According to the figure given by PETERS the dimensions of a normal *Colpidium* is 50 μ length and 16 μ in diameter. Calculated as an ellipsoid the volume works out as 6.7×10^3 μ^3. One liter contains as a maximum 10^7 individuals or 6.7×10^{10} $\mu^3 = 67$ cmm. corresponding to a weight of at least 70 mg. KALMUS gives the metabolism of one *Paramecium* as 5×10^{-6} cc. O_2 per hour. Assuming the metabolism of the somewhat smaller *Colpidium* as $^1/_5$ of this we get in calories per day for one animal $5 \times 10^{-6} \times 24$ or 1.2×10^{-4}. According to PETERS' curve fig. 3 giving the numbers and average dimensions of the *colpidia*, we would have in the first 10 days an average of 2000 per c.c. with a length of 0.7 or a volume of 0.35 of the normal given above, for the next 10 days 6000 with 0.22 vol., for the third period 6000 with 0.16 and for the last 10 days 3000 with 0.12 corresponding in all roughly to 34×10^6 normal individuals per liter in one day, which should have a metabolism of 4000 calories. This figure agrees sufficiently well with the available energy of 2500 calories to justify the assumption that the organic substances must have become at least nearly exhausted.

animals in pure water and those in the "nutrient" solutions, while the normally fed animals had grown considerably. A large number of the starving animals died off during the experimental period and a somewhat smaller number in the "bioklein", while the mortality of the normally fed animals was very slight or absent. Bock noted that the organic solutions were never quite clear and became distinctly clarified when tadpoles lived in them for 24 hours. He also found many more bacteria in solutions without tadpoles and concluded that the tadpoles would live mainly upon the bacteria growing in the solutions. He showed further that the tadpoles would eliminate suspended particles of Indian ink or carmine from water and concentrate them in their intestine, causing the excrements to become intensively black or red respectively.

Bonnet (1927) repeated the experiments of Bock and Kříženecký on tadpoles and sticklebacks and found that the tadpoles do take up some nutrition from bioklein solutions of 1 g. per liter, though much less than in several of Kříženecký's experiments, while sticklebacks do not. Like Bock he ascribes this to the bacteria which are filtered off and swallowed by the tadpoles, but not by sticklebacks or other carnivorous animals.

Kříženecký and Podhradský fed tadpoles in pure water on bacteria cultivated from bioklein solutions and confirmed that these bacteria were ingested, but apparently they were not digested, and the tadpoles could not be distinguished from the controls starving in pure water. I consider these experiments conclusive, and, for reasons to be given later, I think that Kříženecký's main result, that nutritional substances are taken up and utilized by tadpoles from the solutions employed, must be accepted. This does not prove anything with regard to the views of Pütter, since Kříženecký's solutions as he has himself repeatedly emphasized, are many times more concentrated than they can ever occur in nature. The average utilization observed by Kříženecký and his collaborators is much greater than that found by Bock or Bonnet in apparently identical conditions.

In an investigation published in 1924 (III) Kříženecký and Podhradský found that when dissolved substances like bioklein, peptone + glucose, + saccharose or + glycerine, or even glycerine alone were available along with ordinary particulate food the tadpoles would grow to supernormal sizes, and the authors take this to prove the utilization of the dissolved substances. A similar result was obtained with a dilute hay infusion, but a careful perusal of the paper shows that a direct nutritional effect is extremely improbable. Some unknown growth promoting factor must have been present. In later experiments (XIV, 1928) Kříženecký could not himself repeat these results.

In his 13th communication (1928) Kříženecký studied the effect of his dissolved nutrition in lower concentrations down to those found in natural waters. These experiments have a direct bearing upon our problem and must be more fully described. One series was made with equal parts of peptone + saccharose in the following total concentrations in mg./L. A 1000, B 500, C 250, D 124, E 62. The tadpoles of one control group were fed regularly, while those of another were starved. The experiment was begun on 80 tadpoles in each group. The average initial live and dry weights were determined at the beginning on 60 individuals and were on April 8th 12.44 and 1.55 mg. respectively. The following table gives the results.

	May 2nd			June 6th		
	Live weight mg.	Dry weight mg.	Number analysed	Live weight mg.	Dry weight mg.	Number analysed
Fed	108.64	7.06	48	211.39	15.58	42
A	47.90	2.59	27	..	..	0
B	42.23	2.54	33	81.37	4.06	26
C	39.67	2.17	28	69.50	3.47	23
D	27.40	1.40	30	68.77	2.80	18
E	24.20	1.23	25	60.90	2.33	9
Starved	11.00	0.86	34	..	..	0

The conclusion must be accepted that the larvæ have shown definite growth even on the most dilute of the solutions employed.

A corresponding series with the same concentrations of bioklein showed even greater increases in dry weight, that is from 1.44 mg. to 5.7 mg. in 56 days on 62 mg. bioklein pro liter.

In two final parallel series both begun on April 25th and carried on to May 15th, the concentrations of bioklein were varied from 40 down to 2.5 mg. per liter. In one of these series the results correspond more or less to the preceding, the dry weight increasing during the experimental period even on the lowest concentration of bioklein, but the other gave a very different result; all the experimental animals showed decreases in dry weight from 1.6 down to 1.2 mg. in the solution containing 40 mg. bioklein, while in the lowest concentration the final dry weight was 0.9 and in pure water 0.7 mg. No explanation of this discrepancy was found, and KŘÍŽENECKÝ only concludes that at concentrations from 40 mg. downwards the dissolved substances suffice only for maintenance, but do not allow any growth. In the mind of the writer unexplained discrepancies of this kind create an uneasy feeling that the experimental conditions contain an unknown factor which may have vitiated all the results.

The experiments of MITCHELL and SPÄRCK. MITCHELL (1916) (quoted from SPÄRCK) experimented on oysters (*Ostrea virginica*) and found that 2500 mg. of glucose per liter added to the water in which they lived would increase their content of glycogen and fat. In SPÄRCK's experiments on *O. edulis* (1927) 43 mg. glucose per liter was added to water containing one oyster per liter and it was observed by titration of the water according to HAGEDORN-JENSEN that the percentage decreased in one day to 22 mg. and after two days to 4 mg. A similar decrease from 63 to 15 mg. in two days was observed in a second experiment. The oysters were at a stage of starvation, where they could not keep their shells closed, but the addition of sugar restored that ability, while control experiments with fresh oyster shells in the same water showed only a slight diminution in the sugar percentage due to the activity of other organisms (bacteria).

The experiments of YONGE (1928) and KOLLER (1930), which also show that oysters, several other mussels and *Asterias* can take up sugar from solutions, will be more conveniently discussed in the next section.

The experiments of KROGH constitute an attempt to measure in a more direct way than hitherto the organic substances which fresh water organisms might remove

from the water and to compare their heat of combustion with the metabolism of the animals during the same period. A suitable number of animals were placed in a small container through which a flow of water was provided at such a rate that normally about one third to one half of the oxygen available would be used up. In one group of experiments redistilled water of great purity was employed to which the requisite salts and a small amount of glucose was added. The ingoing and outgoing water was analysed for glucose and oxygen. Special methods were worked out (KROGH 1930) to determine the total quantity of combustible organic material in samples of water, and in several cases these were utilized to check the sugar determinations carried out according to the methods of HAGEDORN-JENSEN. In another group of experiments natural water from a lake or pond was used for the flow through the respiration chamber and the quantity of organic material determined by combustion in the ingoing and outgoing water. The experiments were made on animals which were starved for a suitable period beforehand and should show if any amount of organic material, significant in proportion to the metabolism, were taken up.

Experiments with artificial sugar solutions containing from 5 to 40 mg. glucose per liter were made on mussels (*Dreissensia polymorpha*), daphnia (*D. pulex*), small fish (belonging to the carp family) and tadpoles. In control determinations made on water without added sugar some reduction was observed corresponding usually to 1 mg. glucose pro liter, and this reduction was usually increased in the outgoing water by substances given of by the animals. The increase was variable, but usually amounted to about 1 mg./L. When the reduction values were determined in a preliminary experiment the changes in reduction of the sugar solution on passing the respiration chamber were corrected accordingly, it being assumed that the same amount of reducing substance would be eliminated throughout. Calculated in this way most of the experiments show some absorption of sugar representing about 20 per cent. of the metabolism in the same period[1]). In those cases, however, in which the water was subjected to combustion analysis, it could be shown that no energy became available by absorption, since the combustion value of the outgoing water was always higher than that of the ingoing. The added sugar did not measurably increase the metabolism of the animals.

Experiments with natural water were made on mussels (*Dreissensia*) and fishes (*Leuciscus*) from the lake Furesö and generally with water from the same lake containing about 10 mg. organic combustible substances corresponding to 45 calories per liter. The experiments show in all cases a surplus elimination of organic substances, but this appears to be larger in "artificial" water than in the natural, so that possibly small quantities of valuable material are absorbed, while other combustible but useless substances are excreted. The absorption may amount to 10 % of the metabolism observed in the starving animals. It is to be remembered that according to the experiments referred to on p. 17, the lake water contains only a very small proportion of substances of any value to the organisms.

[1]) In the calculation of the exp. 15.IV.1929 on p. 675 of the paper there is an obvious error. The sugar deficit is not 1.8 mg. per liter, but 0.8 and the calories per hour from glucose therefore 4.3 (instead of 9.6) or 22 % of the total.

The Mechanism of Absorption of Nutrient Substances from a Solution.

The experimental evidence detailed above appears to show that in some cases at least (oysters, tadpoles) nutrient substances can be absorbed from solutions, and when the solutions are fairly concentrated, such absorption may be a significant source of nourishment for the animals in question. Although corresponding conditions are not realized in nature, it will be worth while to consider the mechanism of such absorption theoretically and on the basis of existing evidence.

Pütter repeatedly expressed the opinion that in aquatic animals generally the whole of the external surface and especially the gills must be available for the absorption of any substance in any concentration. There is no experimental basis for this sweeping assumption, and in many cases it can be shown to be incorrect.

There are two essentially different ways in which dissolved substances can become absorbed into living beings. One is by diffusion, that is by the movement of dissolved molecules from a medium where their concentration[1]) is higher into another where the concentration is lower. The other is by an active process involving energy by which a substance is transported from a medium where its concentration is low into another in which the concentration is higher. Processes of this second kind take place with regard to threshold substances in the kidney tubules of higher animals, and there is some reason to believe that they take place in the intestine of animals generally, but otherwise they are on the whole unknown and should not be assumed in any case without adequate proof. Unless such an assumption is made the absorption of dissolved substances like glucose or amino acids from natural waters into the blood of metazoa cannot be explained. They cannot move in by diffusion, because the concentration within the blood must normally be much higher than in the outside medium.

For protozoa and generally for animals without a circulation the case is different; the concentration of any diffusible substance can be kept very low in the protoplasm by chemical combination, and it is quite possible that dissolved substances can become absorbed by diffusion even from very dilute solutions, as seems to be the case in some protozoa (Peters). Absorption in the case of protozoa and bacteria is greatly facilitated by the very large surface in proportion to the volume and by the short distances through which the substances have to pass by diffusion. As mentioned above it is possible, though by no means proved by Pütter's experiments, that the sponges fall into this category.

In a large number of aquatic animals we must postulate a high degree of impermeability for the integuments and especially for the gill membranes, because the internal medium is so different from the external that with fairly permeable membranes such differences could not be maintained. In many cases the impermeability postulated can be shown to exist.

The chitinous integument of arthropods generally possesses low permeability and, as it is non living, active absorption is out of the question. Chitin is permeable for gases but the permeability of a chitin membrane for oxygen was found to be only $^1/_{10}$ of that of muscle and $^1/_{25}$ of that of water (Krogh, 1919). A small animal with an exceptionally

[1]) Strictly speaking the product of concentration and solubility.

thin transparent chitinous membrane like the Corethra larva can obtain sufficient oxygen through its integument, but the larvæ of Culex or Anopheles, which being of about the same size have a somewhat thicker integument, must come regularly to the surface to breathe. It was therefore an unfortunate choice, when Pütter attempted to show that a growing Anopheles larva could not possibly obtain sufficient particulate food (Cf. p. 9). Any absorption of dissolved substances through the cuticle is out of the question; the animal must have obtained all its food through the mouth, and the calculation can only serve to throw doubt on Pütter's calculations generally.

The gills and integument of the crayfish were shown by Krogh (1914) to be practically impermeable to a highly diffusible substance like ethyl urethane, but in other cases (daphnia) urethane penetrates fairly rapidly. Even in such cases a diffusion of organic substances rapid enough to be of any significance quantitatively is very improbable, and in most cases the concentration of the substances in question must be higher in the hæmolymph of the animals than in the outside medium. It must be admitted, however, that in some invertebrates under inanition the concentration of sugar in the hæmolymph may become very low (Hemmingsen, 1924).

In the case of fishes both in fresh water and in the sea the osmotic pressure of the blood is very different from that of the surrounding medium, and this involves an extremely low permeability of the gills even for water. Experiments now in progress in the laboratory of the writer (Keys) show that the gills of the eel are practically impermeable to salts and water. They are permeable of course to gases, but there is no evidence for any active absorption of oxygen. It is therefore practically out of the question that fishes should absorb organic substances through the gills.

For the skin of fishes and frogs a unidirectional permeability, from the outside in, for sugar and other substances was found in experiments by Przylecki and Chomkovič, but the concentrations used were from 10—50 g./L. Even so the quantities diffusing through were very small, and it seems very likely that at lower concentrations the skin would be impermeable, in which case the permeability was probably due to impairment of the vitality of the tissue.

Bonnet worked with iron saccharate in solutions in which he kept tadpoles and sticklebacks for periods up to 120 hours. In solutions of 1 g. down to 50 mg./L. no iron was absorbed through the skin and the sticklebacks would even stand 10 g. per liter, but in solutions of higher concentration (10 g./L. for the tadpoles and 50 g./L. for the sticklebacks) the skin, gills and subcutaneous tissue showed the iron colour in several places after 24 hours or less, and the same was the case when 1 g./L. iron saccharate was dissolved with 10 g./L. sugar, showing that even this concentration of sugar would damage the skin.

In all Bonnet's experiments with tadpoles in iron saccharate and even at the lowest concentration of 50 mg./L. the intestine showed the presence of iron already after 1 hour. It seems legitimate therefore to conclude that the absorption of nutrient substances observed in Kříženecký's and Krogh's experiments must have taken place through the intestine. It is commonly asserted that aquatic animals take in their food without water, but it is evident that this assertion is not generally tenable.

The experiments of KOLLER (1927, 1930) and YONGE (1928) definitely show that also in marine invertebrates like mussels and starfishes, absorption of dissolved sugar takes place through the intestine and not through the gills or integument.

YONGE kept oysters in sea water with 2000 mg. glucose per liter and found that they would absorb a considerable proportion at the rate of about 9 mg. per oyster per hour. When, however, the mouth was effectively plugged with wax and plasticine no absorption whatever took place. When the oysters were kept plugged for a period of 8 days profuse "bleeding" occurs by which millions of leukocytes extrude into the mantle cavity. In this condition oysters would absorb sugar from the solution even when the mouth was effectively plugged. YONGE seems to think that in this case the leukocytes take up the sugar, but it appears just as likely that the integument had become permeable.

KOLLER studied the metabolism of *Mya*, *Mytilus*, *Cardium* and *Asterias* in sea water with and without the addition of 50 to 500 mg. glucose per liter. The higher concentrations produced definite increases in oxygen absorption amounting with 200 mg. to 20 per cent. or more and being maintained for some time after the transference of the otherwise starving animals to pure sea water. On *Mytilus* and *Asterias* the mouth could be plugged and when this was done the metabolism in pure sea water remained unchanged and no increase took place in glucose solutions. These experiments like YONGE's show that the absorption takes place through the intestine.

Summary.

The amount of food necessary for aquatic animals is generally present in the form of organisms and organic detritus. There are animals (certain protozoa and perhaps spongiæ) which may take up in addition dissolved substances from the water, and the possibility that a few animal forms live mainly on dissolved substances cannot be excluded. In the case of the cave fauna and that of the ocean depths the ultimate sources of supply of particulate food are so far unknown.

Dissolved organic substances are present in fairly large quantities, 10 mg./L. or more, with a combustion value of 40 calories per liter or more in most fresh waters. The quantities in sea water appear to be of the same order of magnitude. They include proteins in colloidal solution, and a number of well defined amino acids in very high dilutions. The carbohydrates are partially pentosans and do not seem to be readily assimilable.

The dissolved organic substances appear to be mainly waste products and in certain cases at least they were found very resistent to the attack of bacteria.

The plankton algæ do not secrete carbohydrate, but at least so long as they are able to grow they store the material produced by assimilation in their own bodies.

The experimental evidence brought forward by PÜTTER for the absorption of dissolved organic substances in fishes, actinia, ascidia and copepods is inconclusive.

There is no convincing evidence that any animal takes up dissolved organic substances from natural water in any significant amount, but it is made probable by the experiments of PETERS that certain protozoa can do so.

It is possible according to the experiments of KROGH that higher animals take

up insignificant quantities of dissolved combustible material, but the quantities excreted are always larger.

There is strong evidence from the experiments of KŘÍŽENECKÝ and others that tadpoles, mussels and probably many other animals can absorb organic substances from fairly concentrated solutions and at least for a considerable period thrive and grow without particulate food.

The integument and gills of aquatic animals appear to be generally impermeable to organic substances which are practically always present in higher concentration in the body fluids than in the outside medium. The unidirectional permeability of the skin of fishes and amphibia found in certain experiments is probably an artefact. The experiments of BONNET, KOLLER and YONGE show that the absorption of dissolved nutriment observed in tadpoles, mussels and starfishes takes place through the intestine and not through the integument or gills.

References.

ALLEN, E. J.: A contribution to the quantitative study of plankton. Journ. Mar. Biol. Ass. **12,** 1, 1919.

ATKINS, W. R. G.: The respirable organic matter of sea water. Journ. Mar. Biol. Ass. Plymouth. **12,** 772, 1922.

BENEDICT, MILES, ROTH and SMITH: Human vitality and efficiency under prolonged restricted diet. Carnegie publication. **280,** 1919.

BERG, K. and G. NYGAARD: Studies on the plankton in the lake of Frederiksborg Castle. Danske Vid. Selsk. Skrifter. N. M. Afd. Series 9. **1,** 227, 1929.

— — Studies on the plankton in the lake of Frederiksborg Castle. Mem. de l'Acad. Roy. d. Danemark. Sect. Ser. 9. **1,,** No. 4, 1929.

BIEDERMANN in WINTERSTEIN's Handb. der vergl. Physiol. B. II. 649, 1911.

BIRGE, E. and C. JUDAY: The inland lakes of Wisconsin. The plankton. I. Its quantity and chemical composition. Wisconsin Geol. Survey Bull. No. 64, 1922.

— — Organic content of lake water. Bull. Bureau of Fisheries. **42,** 185, 1926.

BLEGVAD, H.: Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. Rep. Danish Biol. Stat. **22,** 41, 1914.

— On the food of fishes in the Danish waters within the Skaw. Ibid. **24,** 17, 1916.

BOCK, FR.: Zur Frage der Ernährung von Amphibienlarven durch im Wasser gelöste Nährstoffe. Zool. Anz. **61,** 171, 1924.

— Weiterer Beitrag zur Frage der Ernährung von Amphibienlarven durch im Wasser gelöste Nährstoffe. Ibid. **64,** 261, 1925.

BONNET, F. W.: Eenige Experimenten omtrent de theorie von PÜTTER. Proefschrift. Leiden 1927.

BOYSEN-JENSEN, P.: Studies concerning the organic matter of the sea bottom. Rep. Danish Biol. Stat. **22,** 1, 1914.

CHOMKOVIČ, G.: Ueber die Permeabilität der Haut bei Fischen für Lösungen von organischen Nährsubstanzen. Pfl. Arch. **211,,** 666, 1926.

DOMOGALLA, JUDAY and PETERSON: The forms of nitrogen found in certain lake waters. Journ. biol. Chem. **63,** 219, 1925.

GRAN, H. und B. RUUD: Untersuchungen über die im Meerwasser gelösten organischen Stoffe und ihr Verhältnis zur Planktonproduktion. Avhandl. Norske Videnskaps. Akad. Oslo. M.-N. Klasse **1926** No. 6.

GAARDER, T. and H. GRAN: Investigations of the production of plankton in the Oslo fjord. Cons. Int. p. l'Expl. de la Mer. Rapp. et Proc.-Verb. **42,** 1, 1927.

— and R. SPÄRCK: Paper to be published shortly in Mém. d. l'Acad. Roy. Danemark.

Hemmingsen, A. M.: The blood sugar of some invertebrates. Skand. Arch. Physiol. **45,** 204, 1924.
Henze, M.: Bemerkungen zu den Anschauungen Pütter's über den Gehalt des Meeres an gelösten organischen Kohlenstoffverbindungen und deren Bedeutung für den Stoffhaushalt des Meeres. Pfl. Arch. **123,** 487, 1908.
Hunt, O. D.: The food of the bottom fauna of the Plymouth fishing grounds. Journ. Mar. Biol. Ass. Plymouth, **13,** 560, 1925.
Kalmus, H.: Untersuchungen über die Atmung von Paramaicium caudatum. Zeitschr. vergl. Physiol. **7,** 314, 1928.
Kerb, H.: Über den Nährwert der im Wasser gelösten Stoffe. Int. Rev. d. ges. Hydrogr. u. Hydrobiol. **3,** 1910.
Keys, A. B.: The weight-length relation in fishes. Proc. Nat. Acad. Sc. **14,** 922, 1928.
Knörrich: Studien über die Ernährungsbedingungen der Daphnien. Plöner Ber. 1901.
Kolkwitz, R.: Plankton-Membranfilter. Ber. d. Deutsch. Bot. Ges. **29,** 1911.
Koller, G.: Versuche an marinen Wirbellosen über die Aufnahme gelöster Nährstoffe. Zeitschr. vergl. Physiol. **11,** 437, 1930.
Kříženecký, J.: Untersuchungen über die Assimilationsfähigkeit der Wassertiere für im Wasser gelöste Nährstoffe. Biologia Generalis. **1,** 79, 1925.
— XIII. Die Abhängigkeit der Ausnützungsweise der gelösten Nährstoffe von ihrer Konzentration. Z. vergl. Physiol. **8,** 52, 1928.
— XIV. Die wachstumssteigernde Wirkung gelöster Nährstoffe (bei geformter Nahrung) als eine labile Reaktion. Ibid. **8,** 69, 1928.
— und J. Podhrasný: Studien über die Funktion der in Wasser gelösten Nährsubstanzen im Stoffwechsel der Wassertiere. I. Die gelösten Substanzen als einzige Nährstoffquelle und die Rolle der Kohlenhydrate und Eiweisssubstanzen im Stoffwechsel der Froschkaulquappen. Pfl. Arch. **203,** 129, 1924.
— und J. Podhraský: Studien III. Zur näheren Kenntnis der wachstumssteigernden Wirkung im Wasser gelöster Nährsubstanzen unter normaler Fütterung mit geformter Nahrung. Ibid. **204,** 26, 1924.
— und J. Podhraský: Studien XI. Ist die Bakterienflora der Vermittler zwischen den Tieren und den aufgelösten Nährsubstanzen. Z. vergl. Physiol. **6,** 431, 1927.
Krogh, A.: Ethyl urethane as a narcotic for aquatic animals, Internat. Revue f. Hydrobiol. **6,** 42, 1914.
— The rate of diffusion of gases through animal tissues. Journ. of Physiol. **52,** 391, 1919.
— Eine Mikromethode für die organische Verbrennungsanalyse, besonders von gelösten Substanzen. Bioch. Zeitschr. **221,** 247, 1930.
— Ueber die Bedeutung von gelösten organischen Substanzen bei der Ernährung von Wassertieren. Z. vergl. Physiol. **12,** 668, 1930.
— und K. Berg: Ueber die chemische Zusammensetzung des Phytoplanktons aus dem Frederiksborg Schlosssee und ihre Bedeutung für die Maxima der Cladoceren. Internat. Revue d. Hydrobiologie, **25,** 204, 218, 1931.
— and E. Lange: On the organic matter given off by Algae. Bioch. Journ. **24,** 1666, 1930.
— — Quantitative Untersuchungen über Plankton, Kolloide und gelöste organische und anorganische Substanzen in dem Furesee. Int. Rev. d. ges. Hydrogr. u. Hydrobiol. 1931.
Lantzsch, K.: Bemerkungen und Zahlen zur Pütter'schen Hypothese. Biol. Zentralbl. **41,** 122, 1921.
Lebour, Marie: The food of post-larval fish I—II—III. Journ. Mar. Biol. Ass. **11,** 433, 1918, **12,** 22, 1918, **12,** 261, 1920.
— Feeding habits of some young fish. Ibid. **12,** 9, 1918.
— The food of young clupeoids. Ibid. **12,** 458, 1921, **13,** 325, 1924.
— The food of plankton organisms, I—II. Ibid. **12,** 644, 1922, **13,** 70, 1923.
Lipschütz, A.: Zur Frage über die Ernährung der Fische. Z. allg. Physiol. **12,** 59, 1910.
— Die Ernährung der Wassertiere durch die gelösten organischen Verbindungen der Gewässer (Eine Kritik). Ergebn. d. Physiol. **13,** 1, 1913.

Lipschütz, A.: Bemerkungen zur Frage über die Ernährung der Wassertiere. Biol. Zentralbl. **38,** 196, 1918.

Lohmann, H.: Untersuchungen z. Feststellung des vollständigen Gehalts des Meeres an Plankton. Wiss. Meeresunters. Abt. Kiel. N. F. 10, 1908.

— Ueber die Quellen der Nahrung der Meerestiere und Pütter's Untersuchungen hierüber. Internat. Revue d. Hydrobiol. **2,** 10, 1909.

— Ueber das Nannoplankton und die Zentrifugierung kleinster Wasserproben z. Gewinnung desselben im lebenden Zustande. Ibid. **4,** 1, 1911.

Mitchell, P. H.: Nutrition of Oysters. Bull. U. S. Bur. Fish **35,** 1916.

Moore, Edie, Whitley and Dakin: The nutrition and metabolism of marine animals in relationship to (a) dissolved organic matter and (b) particulate organic matter of sea-water, Bioch. Journ. **6,** 255, 1912.

Oehler, R.: Amöbenzucht auf reinem Boden. Arch. f. Protistenkunde, **37,** 175, 1917.

— Flagellaten- und Ciliatenzucht auf reinem Boden. Ibid. **40,** 16, 1919.

Peters, R. A.: The substances needed for the growth of a pure culture of Colpidium colpoda. Journ. Physiol. **55,** 1, 1921.

Petersen, C. G. J. and P. Boysen-Jensen: Valuation of the sea I. Animal life of the sea bottom, its food and quantity. Rep. Danish Biol. Stat. **20,** 1, 1911.

Peterson, Fred and Domogalla: The occurrence of amino acids and other organic nitrogen compounds in lake water. Journ. biol. Chem. **63,** 287, 1925.

Przylecki, St. J.: L'absorption cutanée chez les amphibiens. Arch. internat. d. physiologie, **20,** 144, 1923.

Pütter, A.: Studien z. vergleichen den Physiologie des Stoffwechsels. Abh. d. k. Ges. d. Wiss. Göttingen. 1908.

— Die Ernährung der Wassertiere. Z. allg. Physiol. **7,** 283, 1908.

— Der Stoffhaushalt des Meeres. Ibid. **7,** 321, 1908.

— Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Fischer Jena. 1909.

— Die Ernährung der Fische. Z. allg. Physiol. **9,** 147, 1909.

— Die Ernährung der Wassertiere durch gelöste organische Verbindungen. Pfl. Arch. **137,** 595, 1911.

— Der Stoffwechsel der Aktinien. Z. allg. Physiol. **12,** 297, 1911.

— Der Stoffwechsel der Kieselschwämme. Z. allg. Physiol. **16,** 65, 1914.

— Der Stoffwechsel der Copepoden (zugleich ein Beispiel für die Verwendung der Korrelationsmethode in der Physiologie). Pfl. Arch. **201,** 503, 1923.

— Der Umfang der Kohlensäurereduktion durch die Planktonalgen. Pfl. Arch. **205,** 293, 1924.

— Die Atmung der Planktonbakterien. Pfl. Arch. **204,** 94, 1924.

— Die Ernährung der Copepoden. Arch. f. Hydrobiol. **15,** 70, 1925.

Raben, E.: Ist organisch gebundener Kohlenstoff in nennenswerter Menge im Meerwasser gelöst vorhanden? Wiss. Meeresunters. Abt. Kiel. N. F. 11,1910.

Spärck, R.: Studies on the biology of the oyster. Rep. Danish Biol. Stat. **33,** 59, 1927.

Utermöhl, H.: Untersuchungen über den Gesamtplanktongehalt des Kanarenstroms. Arch. f. Hydrobiol. **18,** 464, 1927.

Woltereck, R.: Die natürliche Nahrung pelagischer Cladoceren und die Rolle des "Zentrifugenplanktons" im Süsswasser. Internat. Revue d. Hydrobiol. **1,** 79, 1908.

Yonge, C. M.: The absorption of glucose by Ostrea edulis. Journ. Mar. Biol. Ass. **15,** 643, 1928.

Zuntz and Loewy: Berl. klin. Wochenschr. **53,** 815, 1916.

II. On the Conditions for the Production of Plankton in the Sea.

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Since the Challenger expedition and HENSEN's plankton-expedition in 1889 it has been known that the productivity of the various parts of the ocean differs very greatly. It is also well known from BRANDT's first investigations in the bay of Kiel, that the quantity of plankton living in an area varies with the seasons, with a minimum in the winter in temperate and cold areas and a maximum in spring.

BRANDT has already discussed the possible causes of these variations or, as we now prefer to say, the limiting factors for the production of the sea.

The temperature can not, or at least not directly, limit the growth of the plankton algae as a whole. Algae can grow, and grow abundantly at all temperatures found in the ocean, from $\div$ 1.5° to above 30°, although the different species may have their optima at different temperatures. The warm seas are as a whole less productive than the colder areas, and BRANDT concluded that high temperature in some indirect way must be unfavorable to the growth of the plankton.

The light must of course limit the photosynthesis of the plants against the deep sea. Living plant cells have been found in very great depths, more than 1000 m. below the surface, but they have most certainly sunk down from the surface layers. The productive zone, where the photosynthesis is sufficient to counterbalance the respiration of the algae is rather shallow. Its limit must vary not only with the geographical latitude and the season, but also with the transparency of the surface layers and the density of the plankton organisms suspended in them.

MARSHALL and ORR (1928, 1930) have by culture experiments found the lower limit of the productive zone, the balance depth, in Loch Striven, from less than 10 m. in winter to 30 m. in summer. From the open sea we have no such observations, but in some cases the oxygen tension at different depths may give a clue. In the North Atlantic about 60° N. lat. I judged it (GRAN 1929) to be at about 50 m. depth during the summer.

In high latitudes the productive season is of course short, but even in the North Polar basin the sunshine is for a short time sufficient to produce a vigorous growth between the ice floes.

As Schimper observed during the Valdivia expedition, the light at the surface can also be too strong especially for the diatoms, which draw their chromoplasts together in the centre of the cell (systrophe), and direct sunshine can be lethal (Marshall and Orr 1928).

The variations in the regional and seasonal distribution of the plankton are so irregular, that they could not be explained from the variations in temperature and light. Of more general features it could be stated that the open ocean is as a rule very poor in relation to the coastal areas, and the tropical seas poorer than the temperate areas. Therefore Brandt proposed his well known theory that the production of the sea must be limited by the supply in the surface layers of the necessary nutritive salts, particularly nitrogen and phosphorus compounds.

The methods to determine these salts in sea water were either not exact enough for the small quantities, or too complicate to allow the collecting of a sufficient number of observations for a statistical comparison with the biological conditions. The colorimetrical methods of Denigès, introduced into oceanography by Atkins and Harvey, have made it possible to test Brandt's theory.

The most important nitrogen compounds in sea water have been proved to be the nitrates. Nitrites and ammonia salts can also be used by the algae, but they are found in very minute quantities or less regularly. Although nitrification is not yet proved to be a process regular and characteristical for the metabolism of the sea, it can indirectly be concluded that this must be the case.

The quantities of nitrate, observed in pure sea water, are between 0 and 700 mg. pr. cbm., reckoned as N. The quantity sufficient for a normal rich growth of diatoms at the coasts of Northern Europe may be 90 mg. pr. cbm. The quantity of phosphate, reckoned as P_2O_5, varies between 0 and 200 mg. pr. cbm., while the quantity present in the surface water of Northern Europe just before a rich growth begins in spring, is about 40 mg. pr. cbm.

Brandt's theory has been confirmed by a series of investigations, showing a good correlation between the concentration of the nutritive salts and the growth of the phytoplankton.

Marshall and Orr (1927) found in Loch Striven that the concentration of phosphates in solution in the surface layers decreased with the increase of the pelagic diatoms, and when the phosphates at the surface were used up the maximum of phytoplankton gave place to a minimum, as the diatoms ceased propagating and sank to the depths.

Exactly similar conditions are to be found at the west coast of Norway (Gran 1930). The rich growth of phytoplankton which regularly occurs in early spring (February—April), consisting chiefly of diatoms, disappears in the course of quite a short time after either the nitrates or phosphates in the surface layers have been used up, and the coastal sea, in the following weeks, becomes very poor in plankton.

Schreiber (1928) demonstrated by his culture experiments that the productivity of North Sea water is limited by the lack of either nitrates or phosphates. During summer and autumn the increase or decrease of the plankton is correlated to the variation in the concentration of either nitrates or phosphates.

Also the results of the Meteor expedition from the south Atlantic show a good correlation between the concentration of phosphate and the density of the phytoplankton in the surface layers (HENTSCHEL and WATTENBERG 1930).

Also the theory of NATHANSOHN (1906) on the distribution of the nitrates and phosphates in the sea has been splendidly proved by the latest investigations. The small quantities continuously washed out from shore must be consumed by the pelagic algae in the surface layers and sink downwards in insoluble form with their dead or dying cells or in the bodies or excrements of the animals eating them. Therefore the compounds of nitrogen and phosphorus must continuously be removed from the illuminated surface layers and accumulated in the deep sea, where they again get into solution.

The supply of nutrient salts in the surface layers can be renewed either from the shore or from the deep sea

1) by upwelling of deep sea water as at the west coast of North America or Africa,
2) by turbulence when strong currents sweep along a bottom with uneven depths, as in Puget Sound or over the Faeroe Bank,
3) by convection, when the density of the surface water is increased by cooling.

The effect of upwelling water on the plankton growth is described from La Jolla, Southern California, by W. E. ALLEN (1928) and MOBERG (1928) and from the west coast of South Africa by HENTSCHEL and WATTENBERG (1930).

Off La Jolla ALLEN found during the whole summer a rich diatom plankton between the depths of 15 and 50 m., with a maximum at about 30 m. Above the productive zone nitrates and phosphates were lacking or very scarce, evidently already consumed on the way upwards, and below 50 m. the light was not sufficient for photosynthesis, while the water contained great quantities of nitrates and phosphates. HENTSCHEL and WATTENBERG's maps of the distribution of phosphate and plankton in the surface layers show great quantities of both along the African west coast. The maps show also, that a part of the plankton produced near the shore floats westwards with the surface current, while the phosphates which have nourished it have already been consumed near the shore.

A good example of the effect of turbulence is given by the conditions in Puget Sound (GRAN and THOMPSON 1930). Here heavy tidal currents run through narrow straits of variable depths, and the surface water is constantly mixed with water from greater depths. The surface waters are rich in phosphates throughout the whole summer (THOMPSON and JOHNSON 1930), the rich diatom plankton propagating continuously from May to September does not reduce the concentration noticeably. On the Faeroe Bank the conditions seem to be similar. PAULSEN (1911) and GRAN (1929) both found a much richer plankton over the bank than outside it.

At the west coast of Norway the surface layers are during summer stratified and quite isolated from the deeper layers. The content of nitrates and phosphates is low and the plankton poor. But during winter convection takes place, as SUND (1929) has shown, and in February—March, when the surface temperature has its minimum, the surface layers are rich on nitrates and phosphates over the whole coastal bank. There

is a correlation between phosphate (and nitrate) content and salinity, as the depth to which the vertical circulation reaches depends upon the increase of the salinity downwards. Therefore the phosphate content of the Atlantic water outside the coastal bank is higher than in the coastal current and in the fjords, as the salinity of the Atlantic water is high and uniform right up to the surface.

But when the diatoms begin to grow in February—March, their first rich growth is limited to the coastal current, while the Atlantic water is still very poor.

I have in earlier publications tried to explain this remarkable contrast by supposing that the coastal current had got a rich supply of nutrient salts from the shore. But this supposition cannot be maintained as far as the phosphates and nitrates are concerned. The quantities of nitrates and phosphates which every year are washed out from the land are quite insignificant in comparison with the great reservoir of the deep sea.

The cause of this difference between the coastal current of Western Norway and the Atlantic water outside may also have a connexion with the general fact that coastal areas on the whole are very much richer than the open ocean.

A factor which must be taken into consideration is the biological difference between the oceanic and neritic species of the plankton algae. The neritic species are stationary at the shores and most of them can survive seasons unfavorable for their propagation as resting spores. Many of them are known as extremely rapid growers, as for instance *Skeletonema costatum*. Their metabolism must therefore also be rapid, and they can grow up to a very rich population from a few cells in two or three weeks, when the resting spores are there. At the Norwegian coast the growth of most of the species really begins every year close to the shore and spreads successively out over the bank, while other species can have their centre of distribution at the edge of the shelf where the strong current brings germs from southern coasts. The successive development of the spring growth on the coastal bank may perhaps be sufficiently explained by taking into account the migration of the few cells surviving the winter and the vertical movements of the surface layers causing a part of the scarce population to sink below the lighted zone.

On the other hand the pure oceanic water is populated with species which under unfavorable conditions are liable to sink to such a depth that they are lost, or the currents may move them to areas where the life conditions are no longer suitable for them, and these may be some of the causes for the "sterility" of the Atlantic water in winter and spring.

Conditions similar to those at the Norwegian coasts are described from the Barents Sea by Kreps and Verjbinskaya (1930). The spring growth of pelagic diatoms at the North Russian coast begins in May—June. It is limited to the coastal current, while the Atlantic current farther north is still very poor in spite of its higher content of nitrates and phosphates. The arctic waters north of the ramifications of the Atlantic current are on the other hand still richer in nitrates and phosphates than the Atlantic water, and here the growth of diatoms is also very rich. In spring the arctic waters with their melting ice behave like coastal water, filled with a dense population of diatoms as soon as the light is sufficient. The resting spores can come from the melting ice, as Nansen has shown

(GRAN 1900), and it is of course possible that it is this fact which makes the Arctic waters so fertile when the ice is melting.

In the North Atlantic ocean, north of the Wyville Thomson ridge, the growth of the plankton in spring does not begin untill May, and its distribution is uneven (GRAN 1915). From Shetland and from the Faeroes north-eastwards surface currents can be traced, carrying neritic and also oceanic diatoms in abundance. Often the richest plankton is found in the border zone between Atlantic and coastal water or between Atlantic and Arctic water, indicating that the conditions for a rich growth are satisfied only by a mixture of waters of different origin. The Atlantic water certainly contains enough of nitrates and phosphates, while the coastal (or polar) water may bring either living cells or some stimulating stuff coming from land and lacking in oceanic water.

In recent years some authors have proved that algae and higher plants may be inhibited in their growth by lack of iron in neutral or alkaline solutions (CARSTEN OLSEN 1929, USPENSKI 1927).

CARSTEN OLSEN found in accordance with BOTTOMLEY (1920) that *Lemna minor* grew badly and got chlorotic in ordinary Knop-solutions with $FeCl_3$ as iron nutriment, but abundantly when soil extract was added to the solution. The soil extract could be replaced by ferricitrate with the same effect. The soil extract evidently contains iron in colloid form or in complex ions which are not precipitated in the alkaline solution, and the same must be the case with the citrate. Also for marine algae sea water with an addition of soil extract has of late years been used in laboratories with great advantage.

These considerations gave me the idea that the rich productivity of the coastal waters might be explained by iron-containing humus-compounds drained out from land. These organic iron compounds would be absorbed by the algae and after a shorter or longer period of circulation in living and dead bodies be decomposed and precipitated as inorganic ferri-compounds. From the Challenger expedition it is well known, that the sediments in great depths as in the central Pacific far from shore to a great extent consist of iron and manganese compounds. If the productivity of the coastal waters is dependent on any factor of a chemical nature acting as a minimum factor, it must be an element which in its circulation does not follow the nitrates and phosphates accumulating in solution in the deep sea and reaching the surface again by vertical circulation of any kind. If such minimum stuffs exist, they must irreversibly go out of circulation in the sea, so that they can only be renewed from land.

Our knowledge of the iron content is rather limited, as the quantities present are so minute that it is difficult to get the reagents sufficiently free from iron. HARVEY (1928) has found from 3 to 6 mg. Fe pr. cbm. He concludes that the iron must be present as trivalent organic compounds. KLEM has made some analyses in connection with our investigations at the coast of Møre, using an improved method after MEYER (BRAARUD and KLEM 1931). He found from 4 to 21 mg. Fe pr. cbm., more near land than out in the Atlantic waters, and more in the surface layers than in the depths, in the Romsdalsfjord or outside Storeggen.

I have tried to come nearer to a solution by some culture experiments and shall here give a preliminary report of two of them.

They were arranged with sea water samples of known plankton content in bottles completely filled and closed with ground glass stoppers, suspended in the sea from a horizontal line. All bottles were suspended under optimal light conditions at a depth of 3 m. To the water in some of the bottles was added iron in one or other compound.

The first experiment was made at Aukra March 1930. The water was taken from the surface at Storeggen (our standard station E) just before the plankton diatoms had begun to grow. It contained only 400 cells pr. 1 of Skeletonema costatum. The iron compounds tried were 1) soil extract, 2) Ferrisaccharate and 3) Oxy-haemoglobine prepared from the blood of dogs at the department of physiology of the University of Oslo, and kindly given me by Professor S. Torup. The soil extract was prepared of good garden soil from the botanical garden, Oslo, boiled for 30 minutes in autoclave at a pressure of 2 atmospheres, with an equal volume of distilled water. The filtered extract was once more sterilised in autoclave. Its iron content was analysed by Klem. 2 c.c. of the extract was added to each bottle of 250 c.c. with sea water to give the sea water an iron content of 10 mg. pr. cbm. Corresponding quantities were added of the other iron compounds. The bottles, 18 in number, were suspended in the sea 6 days, from the 16th to the 22nd of March. The results are shown in Table 1.

Table 1. Culture Experiment 16—22 March 1930.
Original content of Skeletonema costatum 400 cells pr. l., 0,4 pr. c.c.,
Temperature 4—5° C.
Number of Skeletonema pr. c.c. after 6 days exposure in 3 m. depth:

No nutrition	Nitrate	Soil extract	Soil extract + Nitrate.	Fe-Sacch.	Oxy-Hæmog.
0.4	0	1,189	849	0	2.0
0.9	0	987	714	1.6	0.8
0.7		956	885		
		804	719		
		808			
Average 0.7	0	949	792	0.8	1.4

No propagation of any significance had taken place in the bottles where no iron was added, and the Ferrisaccharate and Oxyhaemoglobine gave no effect at all, but in all bottles with soil extract the diatoms had grown enormously. The propagation corresponds to nearly two divisions in 24 hours, or to a daily increase of 300 %.

This stimulating effect of the soil extract must of course not necessarily be caused by its iron content. Its content of nitrogen or phosphorus compounds do not come into the question, as the sea water used for the experiment contained more than enough of nitrate and phosphate.

The effect may be dependent on some organic compounds, or upon siliceous acid in the soil extract. The question can only be solved by further experiments.

During the summer 1930 I made some similar experiments at the biological station

at Friday Harbor of the University of Washington. Most of the experiments were indecisive while the diatoms propagated abundantly in all bottles independently of the added nutrition, either nitrate, phosphate, soil extract or different compounds of iron. So rich is the water of the Puget Sound in nutrition often right up to the surface, because the surface layers and the deep water are constantly being mixed. One experiment represented in table 2, seems to give a positive result, but many more experiments will have to be made before the question can be solved.

Table 2. Culture Experiment Friday Harbor July 14—19th 1930. Original content: *Thalassiosira condensata* 25, *Skeletonema costatum* 7, *Nitzschia seriata* 1 pr. c.c. Temperature about 12°. 3 m. depth.

Added:—	No nutrition	Soil extract	$FeSO_4$	$FeSO_4$ $MnCl_2$	$FeSO_4$ $MnCl_2$ $ZnSO_4$
Thalassiosira condensata.....	14	228	59	227	229
Skeletonema costatum.....	30	949	92	356	369
Nitzschia seriata.....	6	19	7	17	18

Another investigation just finished seems to indicate that the growth of the plankton diatoms is determined by other factors than the concentration of phosphates and nitrates besides light and temperature. Mr. JOHAN T. RUUD brought home from his voyage to the Antarctic with M/S "Vikingen" a series of samples for quantitative determination of the plankton. He has published his physical and chemical data in the Journal du Conseil (RUUD 1930). Table 3 gives an extract of some of them together with some of the biological results.

During the antarctic summer 1929—1930 14 stations were taken in the Weddell Sea at intervals of about a week. It was the leading idea of the investigation that it should be possible to follow the development through the season on the supposition that the whole area is sufficiently uniform to be regarded as a unit. For a preliminary, approximative consideration this supposition may hold.

The table shows in accordance with WATTENBERG's results (HENTSCHEL and WATTENBERG 1930) very high values of phosphate content at all stations. Also of nitrates great quantities were found throughout, to 150 mg. P_2O_5 corresponded about 550 mg. nitrate as N.

At the first 7 stations one single species dominated the plankton almost completely, the oceanic species *Corethron Valdiviae*. A number of other species were observed in the net samples collected at the same stations, but only in small quantities. Only at station 6, situated rather near the Bouvet Island, the plankton had a more neritic character, with *Phaeocystis antarctica* and *Thalassiosira antarctica* dominating.

The table shows that the *Corethron* did not propagate very quickly the first 7 weeks, neither did the phosphate content of the surface layers decrease noticeably, as might have been expected. During the whole of this first part of the season the specific

Table 3. Observations in the Weddell Sea 1929—30.

m	1 27/X	2 3/XI	3 13/XI	4 26/XI	5 2/XII	6 12/XII	7 19/XII	8 27/XII	9 6/I	10 14/I	11 22/I	12 28/I	13 12/II	14 17/II
						σ_t [1])								
1	27.09	26.99	27.12	27.09	27.08	27.23	26.92	26.71	27.03	27.00	26.50	26.69	26.29	25.98
10	27.09	27.05	27.15	27.09	27.08	27.24	26.92	26.71	27.03	27.02	26.53	26.71	26.38	26.04
25	27.12	27.09	27.15	27.09	27.08	27.24	26.92	26.73	27.06	27.11	26.87	26.97	27.11	26.05
40	27.13	27.10	27.15	27.09	27.08	27.25	27.04	26.76	27.47	27.55	27.16	27.35	27.47	27.29
75		27.17	27.25	27.25	27.08	27.57	27.25	27.17	27.66	27.64	27.58	27.55	27.66	27.42
						Phosphate, mg. pr. cbm. (as P_2O_5).								
1	150	150	155	140	145		140	120	125	140	95	90	90	
10	150	150	150	145	145	140	140	130	125	140	95	100	95	90
25	150	150	150	145	145	140	145	130	130	145	105	100	130	100
40	150	150	150	150	150	140	145	140	150	150	135	140	145	150
75	150	150	150	150	150	140	145	150	150	160	155	165	150	150
						Phosphate, mg. pr. cbm., consumed.								
1	..	..	..	10	5		10	30	25	10	55	60	60	
10	..	..	..	5	5	10	10	20	25	10	55	50	55	60
25	..	..	..	5	5	10	5	20	20	5	45	50	20	50
40	..	..	..	..	..	10	5	10	..	..	15	10	5	..
75	..	..	..	..	..	10	5	..	..	..	..	..	..	..
						Diatoms, total number, cells pr. c.c.								
1	..	1	..	2	1	28	2	7	28	2	3	32	16	11
10	..	..	..	1	1	15	4	9	10	2	9	119	4	12
25	..	1	..	1	2	13	1	16	27	1	19	15	9	26
40	1	1	..	2	1	22	1	9	4	1	22	25	1	1
75	..	2	..	1	2	7	1	1	..	..	4	4	..	3
						Corethron Valdiviae Karsten, cells pr. l.								
1	220	160	180	1,680	480	40	1,680	800	40	..	40	..	..	..
10	20	140	80	940	100	..	3,420	1,940	40	40	..	100	..	..
25	340	140	250	730	450	40	240	2,120	430	..	20	..	..	..
40	850	100	100	1,140	560	..	60	920	..	20	20	..	..	..
75	60	320	100	190	410	..	..	520	20	60	20	20	..	20
						Thalassiosira antarctica Comber, cells pr. l.								
1	..	..	..	40	..	3,160	40	20	2,060	260	140	8,240	..	..
10	..	..	..	..	..	5,280	..	180	1,480	200	380	11,060	..	..
25	..	..	..	60	..	5,580	..	140	3,120	..	5,280	1,280	80	20
40	..	..	20	..	..	4,740	80	100	360	220	3,060	4,700	100	100
75	40	..	40	120	..	320	..	..	..	..	120	760	120	..
						Rhizosolenia bidens Karsten, cells pr. l.								
1	10	..	20	..	..	..	..	1,470	880	40	..	80	..	..
10	..	..	..	..	20	..	20	690	970	..	..	20	..	..
25	..	..	..	20	..	..	..	1,680	990	20	30	10	80	..
40	20	..	..	..	20	..	..	870	410	20	380	10	..	..
75	..	..	..	..	..	10	30	50	..	..	..	..	..	..
						Chaetoceros atlanticus Cleve, cells pr. l.								
1	..	..	..	..	..	..	..	..	100	..	460	3,760	6,180	1,560
10	..	..	..	..	..	..	..	..	..	..	2,240	73,400	2,340	3,960
25	..	..	..	..	..	..	..	..	..	..	2,580	160	2,240	11,240
40	..	..	..	..	..	..	..	..	..	..	5,280	20	40	20
75	..	..	..	..	..	..	..	..	..	..	..	..	60	80

[1]) A horizontal line below the figures indicates an increase of 0.20 or more to the next depth downwards.

gravity (σ_t) of the surface layers was remarkably uniform, indicating vertical circulation. Also the *Corethron* cells are more evenly distributed from the surface to 75 m. or more, which is different to what one is accustomed to find during the growth period of the diatoms. My explanation is that the diatoms also move with the vertical movements of the water, and that therefore no accumulation is found in the illuminated zone, with the effect that the whole production is retarded, because too many of the diatoms sink below the balance depth of the photosynthesis, in this area probably a depth of about 50 m.

From about the new year the situation shows a change. The specific gravity shows a remarkable increase downwards right from the surface, a "sprungschicht" is formed at station 9—10 between 25 and 40 m. and at station 11—13 even between 10 and 25 m. This change must be caused by the melting of the ice. It has the effect that the vertical circulation is stopped and the sinking of the diatoms retarded, and now it is clearly seen, how the phosphates (and nitrates) are consumed in the surface layers as far down as the photosynthesis is effective. At a depth of 40 m. consumption is already strongly reduced. (These observations are sufficient to show that SCHIMPER and KARSTEN gave a wrong explanation of the observations made during the Valdivia expedition, when they concluded from the vertical distribution of the plankton diatoms that they had their optimal conditions at a depth of about 40 metres).

From the middle of January *Thalassiosira antarctica* (and some other neritic species, e. g. *Biddulphia striata* and *Eucampia Balaustium*) began to form resting spores and sink, and by the 12th of February they had practically disappeared. And still less than half of the nitrates and phosphates were consumed, and the changes in temperature and salinity were quite inconsiderable. Some of the oceanic species, e. g. *Chaetoceros atlanticus*, still continued propagating, but they too showed a tendency to sink, and the oxygen tension in the surface layers began to decrease from station 13 (February 12th). It may be maintained that the growth of the diatoms here was never stopped by lack of phosphates or nitrates.

Further investigations may show what the limiting factors may be.

References.

1914. ALLEN, E. J.: On the culture of the Plankton Diatom Thalassiosira gravida Cleve, in Artifical Sea-water. Journal of the Marine Biological Association. Vol. 10.

1928. ALLEN, W. E.: Catches of Marine Diatoms and Dinoflagellates taken by boat in Southern California Waters in 1926. Bulletin of the Scripps Institution of Oceanography La Jolla, California. Technical series, Vol. 1, No. 13.

1923. ATKINS, W. R. G.: The phosphate content of fresh and salt waters in its relationship to the growth of the algal plankton. Journal of the Marine Biological Association. Vol. 13.

1920. BOTTOMLEY, W. B.: The Growth of Lemna Plants in Mineral Solutions and in their Natural Medium. Annals of Botany, Vol. XXXIV, p. 345.

— The Effect of Organic Matter on the Growth of various Plants in Culture Solution. Ibidem p. 353.

1931. BRAARUD, TRYGVE and KLEM, ALF: Hydrographical and Chemical Investigations in the Sea off Møre and in the Romsdalsfjord. Skrifter utgitt av Hvalraadet ved Universitetets Biologiske Laboratorium, Oslo. Nr. 1 (in print).

1899. Brandt, K.: Ueber den Stoffwechsel im Meere. Wissenschaftliche Meeresuntersuchungen. N. F. Abt. Kiel, Bd. IV.
1909. Chun, Carl: Die deutsche Tiefsee-Expedition 1898—99. Zeitschrift der Gesellschaft für Erdkunde zu Berlin, 34.
1900. Gran, H. H.: Diatomaceae from the Ice Floes and Plankton of the Arctic Ocean. The Norwegian North Polar Expedition 1893-1896. Scientific Results edited by Fridtjof Nansen, Vol. IV.
1915. — The Plankton Production of North European Waters in the Spring of 1912. Bulletin Planctonique pour l'année 1912, publié par le Bureau du Conseil.
1929. — Quantitative Plankton Investigations carried out during the Expedition with the "Michael Sars", July—Sept. 1924. Rapports et Procès-Verbaux Vol. LVI.
1930. — The spring growth of the plankton at Møre in 1928—29 and at Lofoten in 1929 in relation to its limiting factors. Skrifter utgitt av Det Norske Videnskaps-Akademi i Oslo. I. Mat.-Naturv. Klasse. 1930. No. 5.
1930. Gran, H. H. and Thompson, Thomas, G.: The Diatoms and the physical and chemical conditions of the sea water of the San Juan Archipelago. Publications Puget Sound Biological Station. Vol. 7.
1926. Harvey, H. W.: Nitrate in the Sea. Journal of the Marine Biological Association. Vol. 14.
1928. — Biological Chemistry and Physics of Sea Water. Cambridge University Press.
1930. Hentschel, Ernst und Wattenberg, Hermann: Plankton und Phosphat in der Oberflächenschicht des Südatlantischen Ozeans. Annalen der Hydrographie, LVIII.
1905. Karsten, G.: Das Phytoplankton des Antarktischen Meeres nach Material der Deutschen Tiefsee-Expedition 1898—99. Deutsche Tiefsee-Expedition 1898—99, Bd. II, 2. Teil.
1930. Kreps, E. and Verjbinskaya, N.: Seasonal Changes in the Phosphate and Nitrate Content and in Hydrogen Ion Concentration in the Barents Sea. Journal du Conseil, Vol. V, p. 329.
1927. Marshall, S. M. and Orr, A. P.: The relation of the Plankton to some Chemical and Physical Factors in the Clyde Sea Area. Journal of the Marine Biological Association. Vol. 14.
1928. — The Photosynthesis of Diatom Cultures in the Sea. Ibidem. Vol. 15.
1930. — A Study of the Spring Diatom Increase in Loch Striven. Ibidem Vol. 16.
1928. Moberg, Erik A.: The Interrelation between Diatoms, their chemical Environment, and Upwelling Water in the Sea, off the coast of Southern California. Proceedings of the National Academy of Sciences. Vol. 14. No. 7.
1906. Nathansohn, A.: Ueber die Bedeutung vertikaler Wasserbewegungen für die Produktion des Planktons im Meere. Abh. der Math. Physischen Klasse der Kgl. Sächs. Ges. der Wissenschaften. Bd. 29. Nr. 5.
1929. Olsen, Carsten: Om Humusstoffernes Indflydelse paa Væksten af grønne Planter i Vandkultur. Meddelelser fra Carlsberg Laboratoriet, Bd. 18, Nr. 1.
1909. Paulsen, Ove: The Plankton of a submarine bank. Biologiske Arbeider, tilegnede Eug. Warming, 3. Nov. 1911.
1930. Ruud, Johan T.: Nitrates and Phosphates in the Southern Seas. Journal du Conseil. Vol. V.
1928. Schreiber, Ernst: Die Reinkultur von marinem Phytoplankton und deren Bedeutung für die Erforschung der Produktionsfähigkeit des Meerwassers. Wissenschaftliche Meeresuntersuchungen. Abt. Helgoland, XVI.
1929. Sund, Oscar: The Determination of Nitrates in Sea Water. Rapports et Procès-Verbaux, Vol. LIII.
1930. Thompson, Thomas G. and Johnson, Martin W.: The sea water at the Puget Sound Biological Station from September 1928 to September 1929. Publications Puget Sound Biological Station, Vol. 7, p. 345.
1927. Uspenski, E. E.: Eisen als Faktor für die Verbreitung niederer Wasserpflanzen. Jena. Pflanzenforschung, herausgegeben von Prof. Dr. R. Kolkwitz.

III. Biochemical and Biological Investigations of the Variations in the Productivity of the West Norwegian Oyster Pools.

By

Torbjörn Gaarder and **R. Spärck.**

The oyster pools or oyster basins of Western Norway are characterised by extraordinary conditions of production. The summer temperatures are much higher than usual at our latitudes, and in summer the water layers, in which the production of plankton and oyster larvae goes on, are cut off from the sea outside and — because of the brackish surface layer — also from the air. The productive water layers are so to speak a separate world — a microcosmos. We therefore thought it would be of a certain interest to study these peculiar conditions of the oyster pools, on account of their importance to the problems of production in the sea as well as to the practical results of oyster culture.

In 1925 R. Spärck therefore undertook a preliminary investigation of some oyster pools, and later on, in 1927, together with Dr. Gaarder of Bergen, started, in the Espevik pool, Hardanger, a more thorough investigation which was continued in 1928 and 1929. We shall in this paper give only a small record of these investigations[1]) which were financed by the Zoophysiological Laboratory of the University of Copenhagen. We are much indebted to Professor A. Krogh, Director of the Laboratory, for his great interest in our work.

To begin with we shall say a few words about the oyster pools. An oyster pool is a little inlet, more or less separated from the sea with which connection only exists at high tide. As already mentioned in the paper by Helland-Hansen this causes the development of a brackish surface layer especially when the connection with the sea is inconsiderable. Because of the great variations in the salinity of the water outside, more salt sea-water sinks to the bottom during winter-time, whereas the surface layer, which consists of the more brackish water, during summer-time becomes still more brackish on account of the precipitation. And this surface layer further implies, as stated by Amund Helland, the high summer temperatures of the deeper water.

Our method has been the following: with intervals of a few days to make de-

[1]) More detailed discussions will be published later in Bergens Museums Aarbok. Bergen (the hydrographical and biochemical part) and in another paper (the biological part) published in Copenhagen.

Fig. 1. The Espevik-pool on the island Tysnes, S. of Bergen. (Phot. by T. Gaarder). The pool (in the foreground) and the open fjord (in the background) are seen connected by a narrow channel, which is closed in summer-time.

terminations of salinity, temperature, oxygen, pH, calcium, nitrate, phosphate, oxidizability, nannoplankton, and then comparisons between these hydrographical-biochemical conditions and the state of the zooplankton and that of the oysters.

The temperature of the oyster pools is mainly known from the investigations of Helland-Hansen. Because of the sunshine the water is in the deeper part heated and as the deeper layers are separated from the air through the brackish surface layer, it cannot cool down in the night in periods without sunshine. This is what causes the high temperatures. In the three summers in question the maximum temperatures at 1—2 m. were the following:—

1927.....	25—26° C.
1928.....	21—22° C.
1929.....	26.8° C.

The content of oxygen was very different in these three years, the maximum content at 1—2 m. was the following:—

1927..... 11—12 cc. per l.
1928..... 7.3 cc. - l.
1929..... 11—12 cc. - l.

Whereas the content in 1927 and 1929 was very high and increasing in the first part of the summer, it was in 1928 much lower and not increasing. As an uptake of oxygen from the air is totally excluded below the brackish surface layer the high and increasing content of oxygen must have been produced in the water of the pool, which

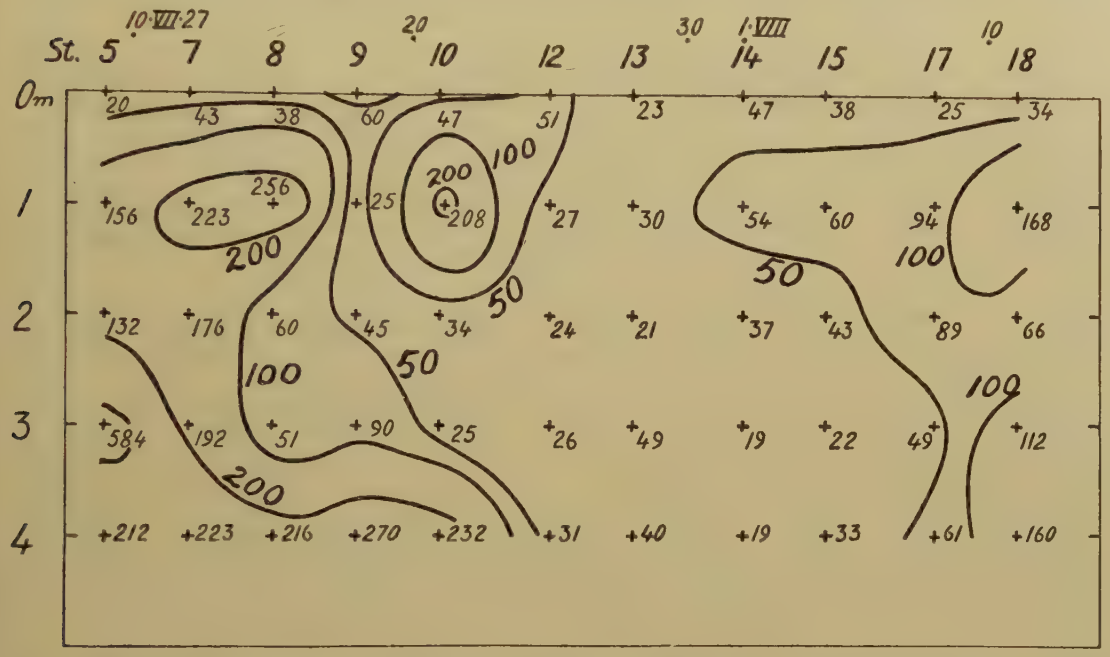

Fig. 2. Number per mm³ of micro-organisms of the size-category 1—2 μ in the Espevik-pool in the summer 1927.

indicates that a very rich production of assimilating organisms, producing more oxygen than was necessary for respiration, must have taken place.

In 1928, on the contrary, the production of oxygen and the demand for it were about equal.

The pH determinations show a very close relation to the content of oxygen.

In 1927 the maximum of pH was ..... 8.7—8.9.
- 1928 — — 8.3
- 1929 — — ..... 8.7—8.8.

The determinations of nitrate-phosphate gave the result that these compounds were present in such small quantities, that it was not possible to determine them with the colorimetric methods of Atkins and Harvey.

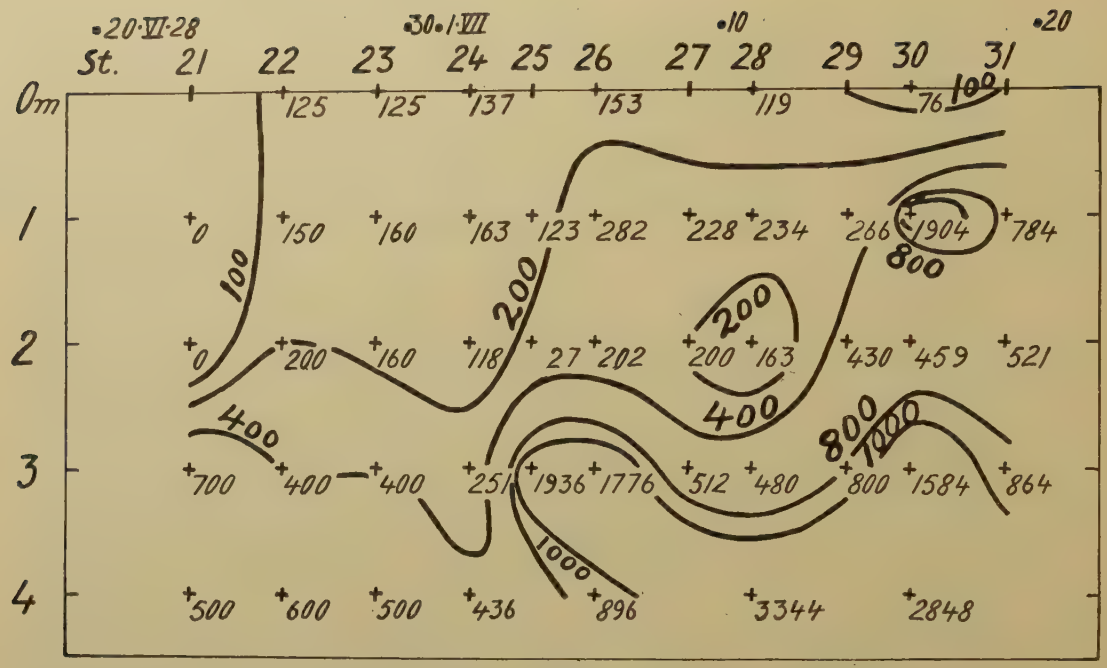

Fig. 3. Number per mm³ of organisms of the size-category 1 μ in the Espevik-pool in the summer 1928.

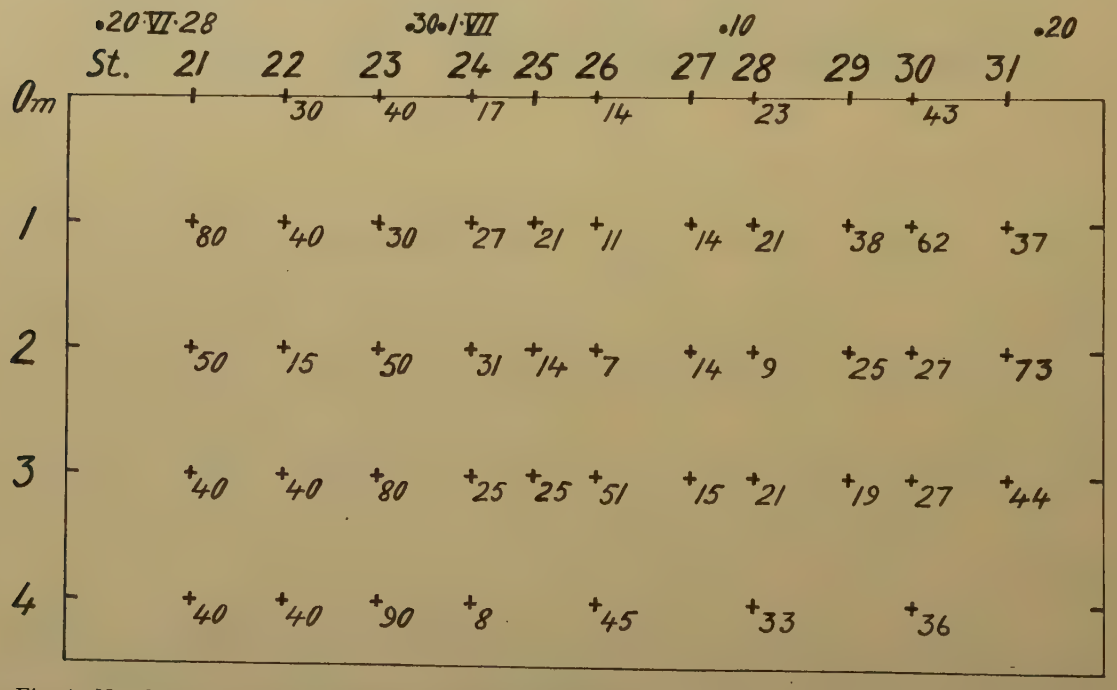

Fig. 4. Number per mm³ of organisms of the size-category 2 μ in the Espevik-pool in the summer 1928.

We shall now compare the hydrographical factors with the content of plankton. Fig. 2 demonstrates the content of small organisms (number per mm.³) in the Espevik pool in the summer 1927. It will be seen that there is a maximum (a little more than 200 per mm.³) in the upper part of the salt-water layer, the most productive part of the water layers. This maximum consists of *Chlamydomonas mikroplankton* and allied, assimilating green organisms. In the deeper part of the pool, at a depth of 3—4 m., there is another maximum of small organisms, here consisting of Bacteria, and therefore decreasing in the course of summer on account of the influence of the sunshine, and reappearing at the end of August. Fig. 3 demonstrates the content of Bacteria in the

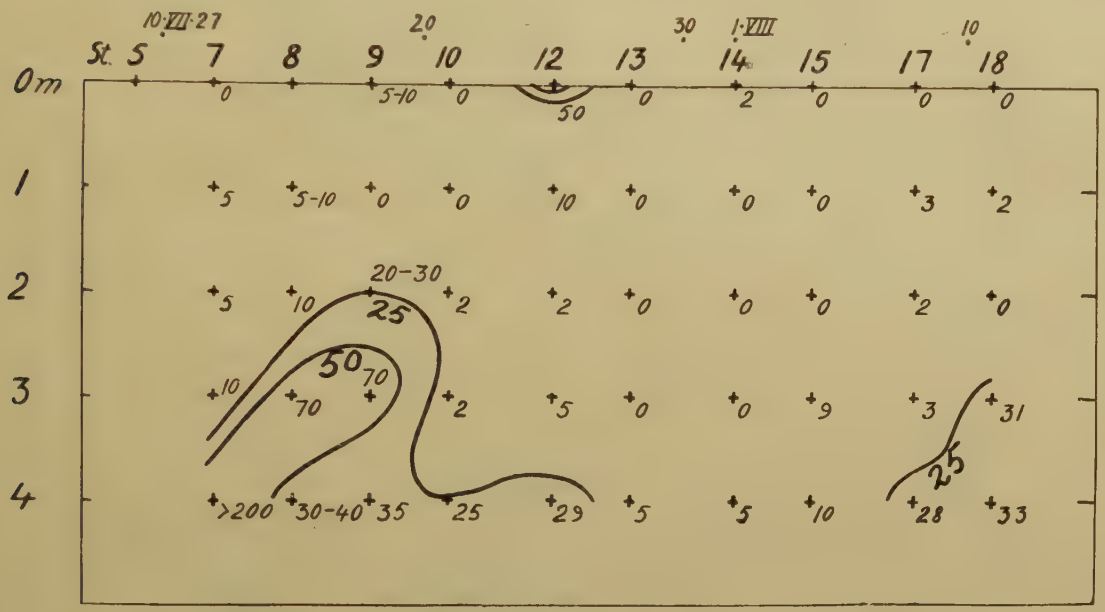

Fig. 5. Number per 10 l of *Peridinea* in the Espevik-pool in the summer 1927.

summer 1928. It will be seen that the number in this summer is much higher (maximum is more than 1000 per mm.³) and that Bacteria commonly occur also in the upper part of the salt-water layer. The number of assimilating organisms (fig. 4) this summer was, on the contrary, very low, only in one or two days reaching numbers of more than 50 per mm.³.

From the graphs on figs. 5—7 the number of *Peridinea* per 10 l. will appear. In 1927 only a few *Peridinea* per 10 l were found, and especially in the deeper part of the pool, not in the productive part. In 1928 *Peridinea* were quite dominant in the pool from surface to bottom in numbers of several thousands per 10 l. And in 1929 *Peridinea* were rather common in the brackish surface layer and in the deeper part of the salt-water layer, but they were very scanty in the really productive part of the pool between 1 and 3 m.

From the graphs on figs. 8—10 the content of gastropod larvae (probably larvae

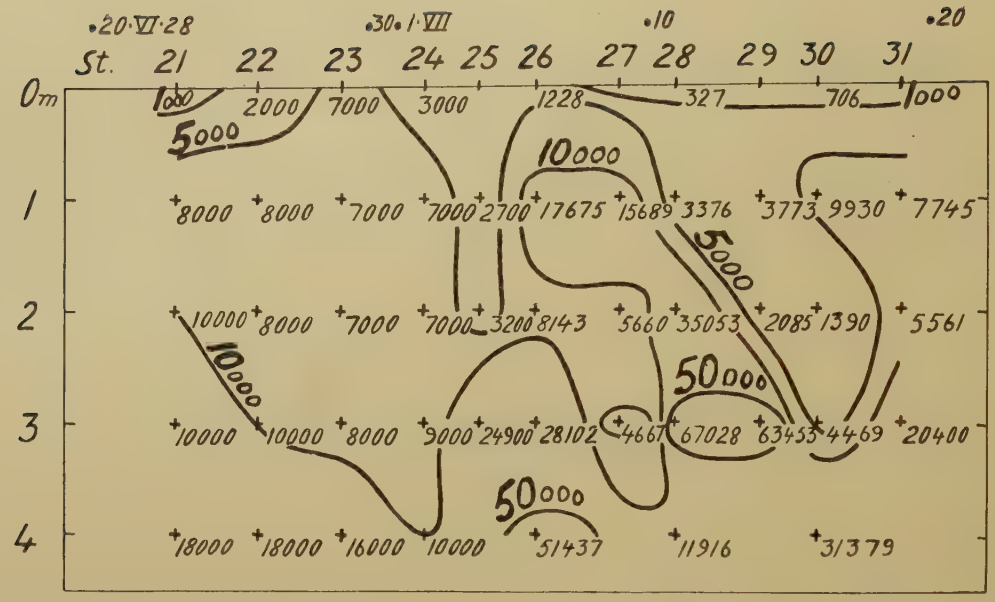

Fig. 6. Number per 10 l of *Peridinea* in the Espevik-pool in the summer 1928.

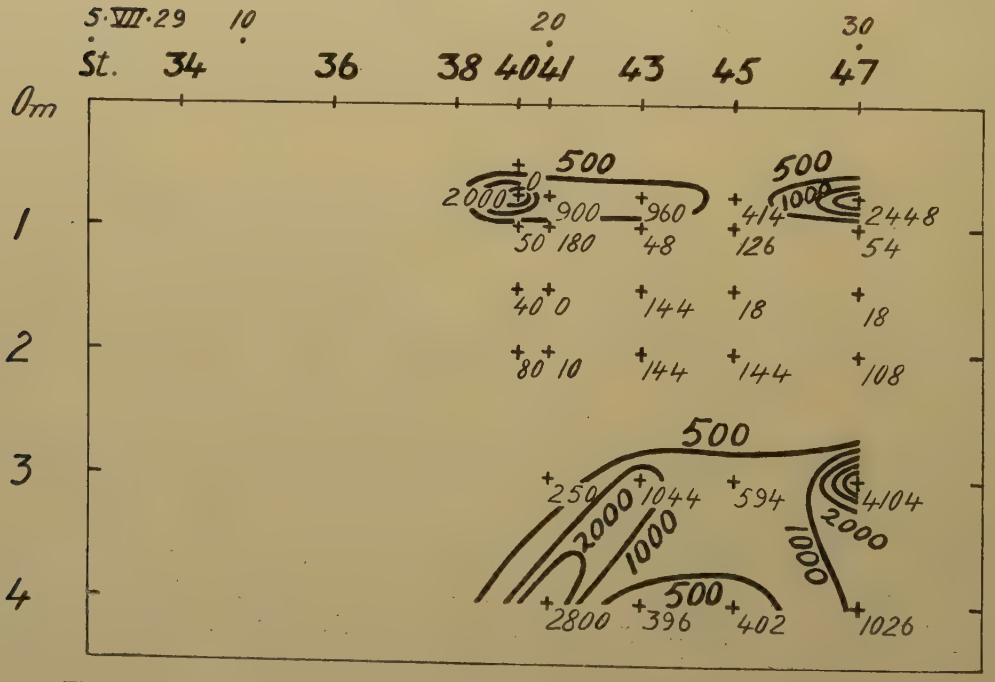

Fig. 7. Number per 10 l of *Peridinea* in the Espevik-pool in the summer 1929.

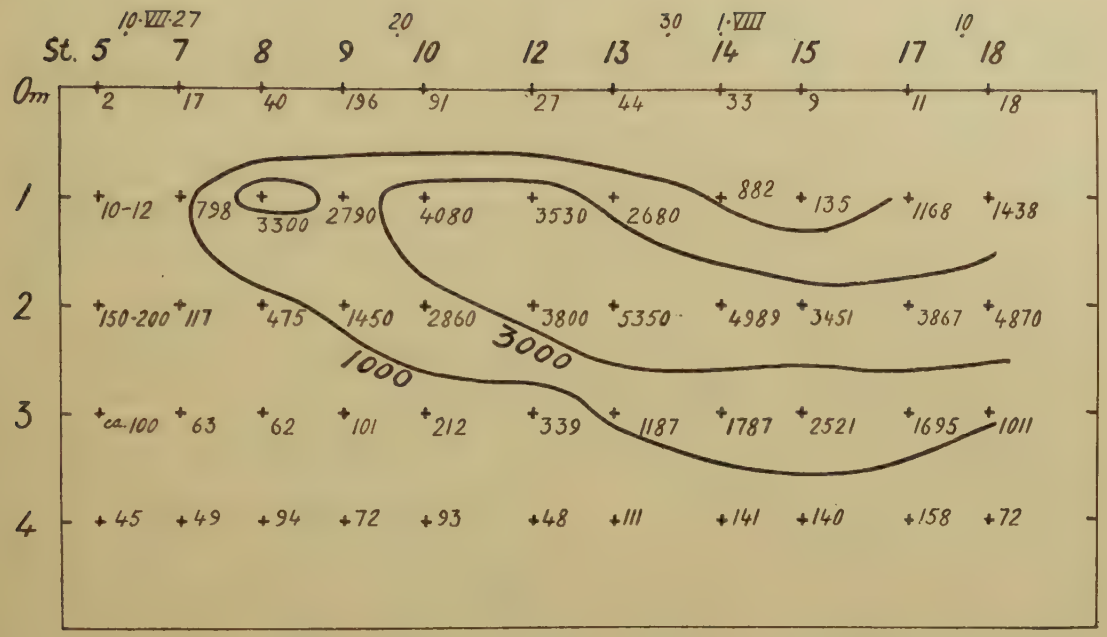

Fig. 8. Number of Gastropod-larvae per 10 l in the Espevik-pool in the summer 1927.

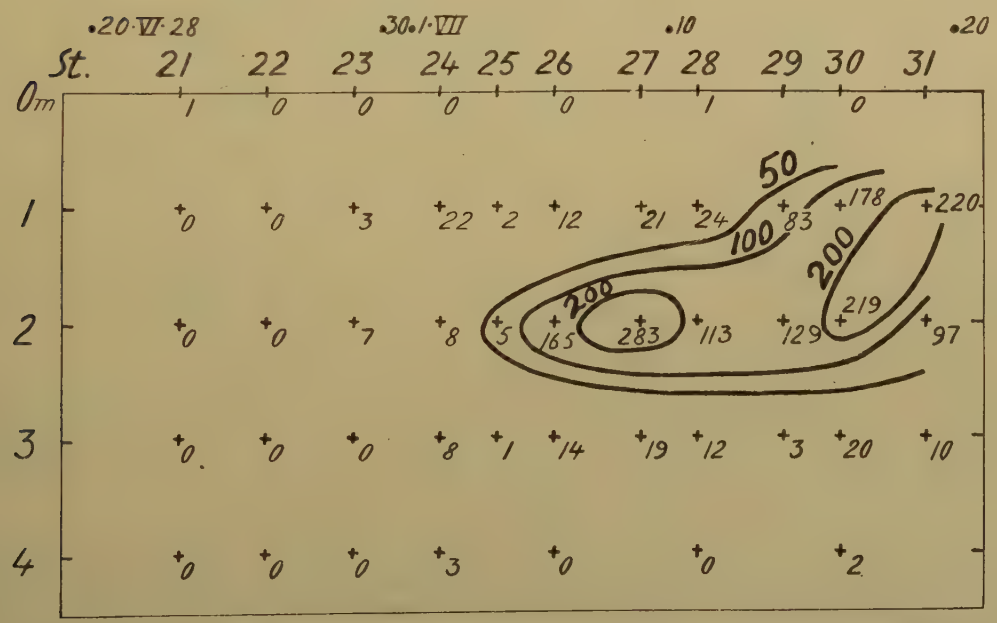

Fig. 9. Number of Gastropod-larvae per 10 l in the Espevik-pool in the summer 1928.

of *Cerithium*) will appear as an example of a zooplanktonic organism. In 1927 and 1929 there was a great amount of gastropod larvae (more than 1000 per 10 l), whereas the number in 1928 was much smaller, and the occurrence of the larvae was restricted to a much narrower water layer.

If finally we look at the graphs on figs. 11—13, it will be seen that also as regards the oyster larvae quite the same is the case. In 1927 and 1929 there was a number of larvae (20—50 pr. 10 l) in the productive water layers. In 1927, the year with the most sunshine, oyster larvae were present at depths of from 1—4 m, in 1929 with

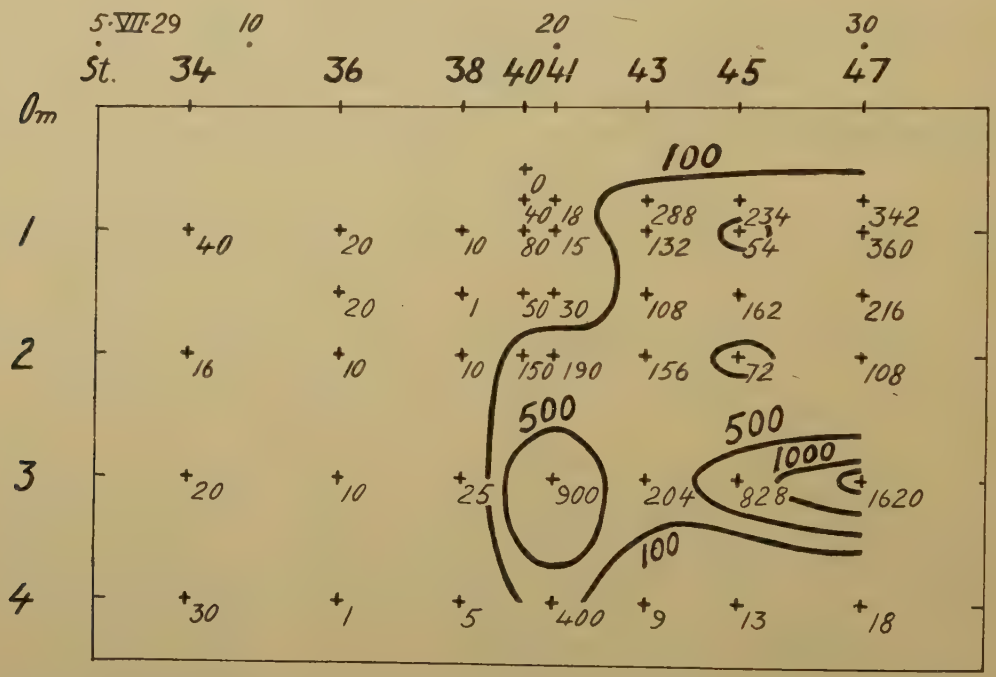

Fig. 10. Number of Gastropod-larvae per 10 l in the Espevik-pool in the summer 1929.

less sunshine, the oyster larvae were only found in the upper part of the salt-water layer, at depths of about 1—2 m. On the other hand, in 1928, with the summer almost without sunshine, practically no oyster larvae were found.

It may further be noticed that in 1928 also the adult oysters in the pool died in the course of June—July, especially the old individuals. As the salinity and the content of oxygen were sufficiently high, this mortality must have been due to starvation, and it is of interest to notice that even the adult oysters were not able to feed on Bacteria and Peridinea for longer periods at temperatures of about 21° C.

The result of this comparison is that in 1927 and 1929, the years with much sunshine, we had an enormous production of assimilating organisms, indicated through the content of oxygen and the high value of pH. The main part of the producing organisms was quite small nannoplanktonic organisms of a size-category of 1—3 μ, whereas the greater

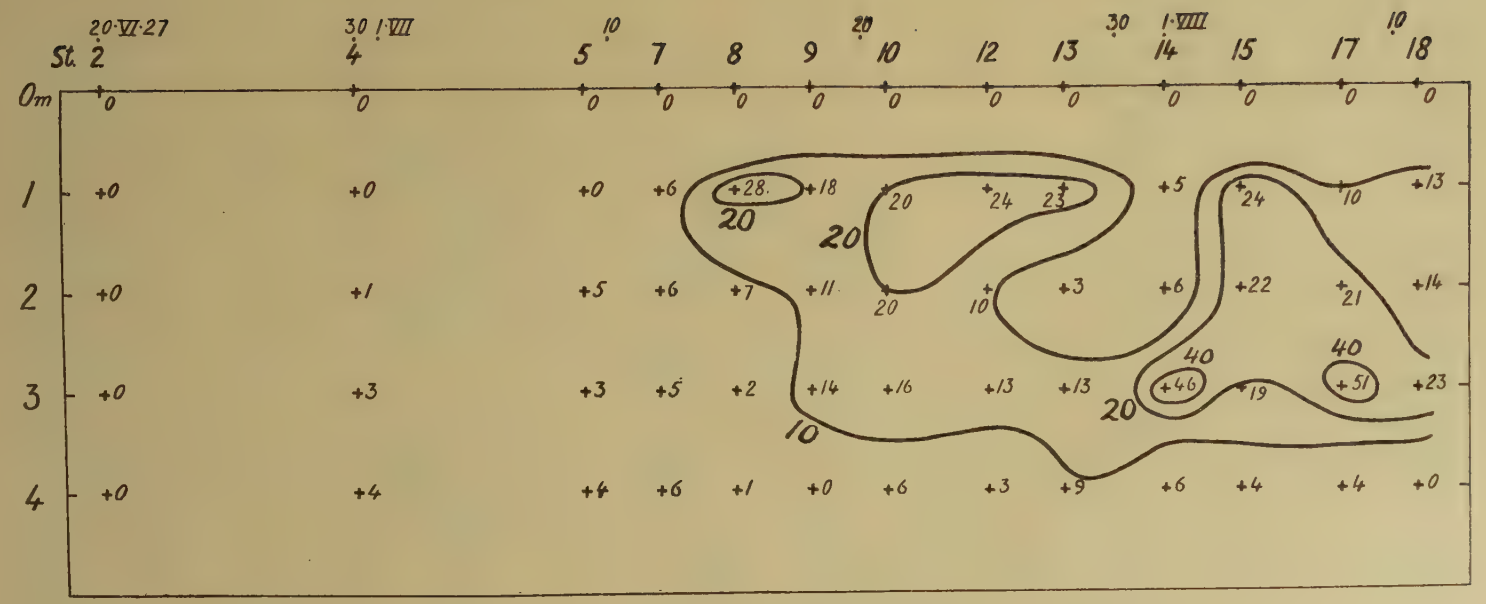

Fig. 11. Number of oyster-larvae per 10 l in the Espevik-pool in the summer 1927.

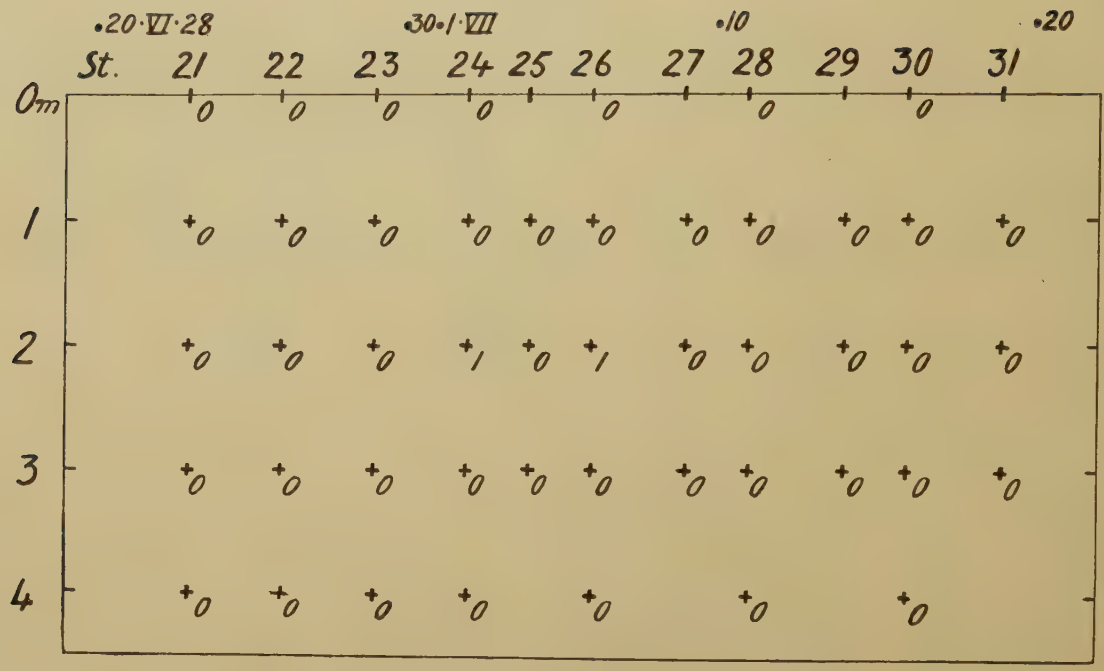

Fig. 12. Number of oyster-larvae per 10 l in the Espevik-pool in the summer 1928.

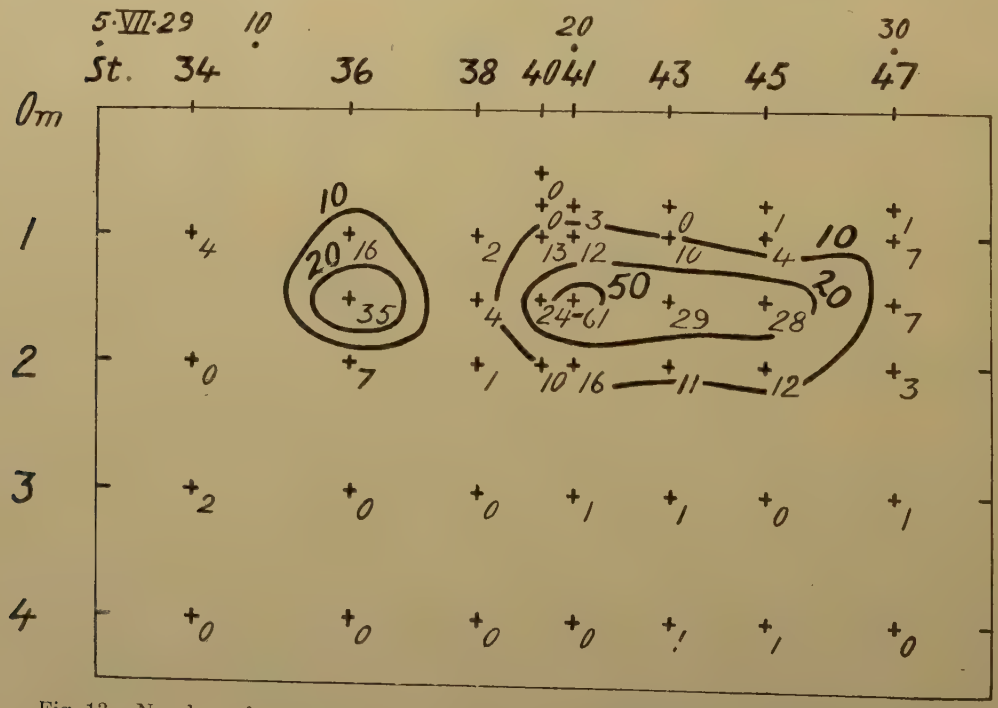

Fig. 13. Number of oyster-larvae per 10 l in the Espevik-pool in the summer 1929.

phytoplanktonic organisms as for instance Diatomea were of hardly any importance. These small green organisms served as food to the zooplankton, for instance the oyster larvae, as well as to the oyster itself, and the production of these small organisms consequently implied a high production of zooplankton.

In 1928 the content of Bacteria and Peridinea in the plankton was on the contrary prevalent, and the zooplankton was lacking. These organisms are consequently useless as a sole food. The main conclusion is that the production of assimilating organisms in the sea-water itself is the really productive factor in the oyster pools and constitutes the direct nourishment for the animals, which organic substances washed out from the surrounding do not. In 1928 — which was a very rainy year — much detritus and organic substances were washed out from the sourrounding land into the pool, but nevertheless the food conditions for the animals were utterly bad.

Table I.

Depth	S ‰	t°	p_H	cc. O_2 per liter sea-water					
				At the beginning	After 72 hours				
					without adding nitrate-phosphate	Difference	nitrate-phosphate added	Difference	dark
1 m.	26.34	18.9	8.30	7.30	6.78	—0.52	7.46	+0.16	5.68
2 -	30.00	18.8	8.28	6.61	6.22	—0.39	6.63	+0.02	5.98
3 -	30.23	17.0	8.19	6.32	6.27	—0.05	6.87	+0.55	5.64
4 -	30.23	15.8	7.87	3.30	2.43	—0.87	2.52	—0.78	1.94

Table II.

Depth	O_2 produced per l		Difference	O_2-uptake per liter
	without adding nitrate-phosphate	nitrate-phosphate added		
1 m.	1.10	1.78	+0.68	1.62
2 -	0.24	0.65	+0.41	0.63
3 -	0.63	1.23	+0.60	0.68
4 -	0.49	0.58	+0.09	1.36

The factors regulating the productivity of the green algæ are the sunshine and the supply with nitrate-phosphate. It is not possible for us to regulate the sunshine, but as regards the content of nitrate-phosphate we may, however, do something. Our culture experiments after the method of Gaarder and Gran (e. g. Table I—II) have shown that in years with few hours of sunshine the productivity can be increased by adding

nitrate-phosphate to the sea-water. In years with much sunshine this addition is superfluous, because the production is so accelerated that even a very small but unceasing supply with nitrate-phosphate can be very impressive to the assimilating organisms. In 1929 we made an attempt to increase the production in the Espevik pool where the conditions were for several reasons rather bad in the spring. 0.1 mg. per l of nitrate-N and phosphate-P were added and the production was clearly increased.

The practical value of the Norwegian oyster pools has hitherto been rather small, on account of the strongly varying spat fall. It is possible e. g. that through adding nitrate-phosphate we may be able to regulate these variations, and this will be of importance not only to the oyster culture in Norway but perhaps also to the production of oyster spat all over Europe, where the demand for this is nowadays very great.

IV. Eine biologisch chemische Studie im Hafenwasser von Helsingfors.

Von

Kurt Buch.

Institut für Meeresforschung, Helsingfors, Finnland.

Vorliegende Studie hatte die Aufgabe zu untersuchen, ob und welche Fluktuationen sich in den von einander abhängigen chemischen und biologischen Faktoren im Wasser unter den günstigen Bedingungen eines intensiven Stoffwechsels während des Tagesverlaufes erkennen lassen. Als Untersuchungsobjekt wurde ein inneres Hafengebiet von Helsingfors, die Tölöwiek, gewählt, welche von Abwasser stark verunreinigt und daher infolge ihres Reichtums sowohl an mineralischen wie organischen Nährstoffen ein üppiges sowohl pflanzliches wie tierisches Planktonleben aufweist. Besonders bestand die Hoffnung, die von der Stärke der Belichtung abhängige Assimilationstätigkeit zu verschiedenen Tageszeiten näher verfolgen zu können.

An der Untersuchung waren zwei Personen beteiligt: Doktor I. Välikangas, der den biologischen und Verf., der den chemischen Teil übernommen hatte. Herr Välikangas hat noch nicht Gelegenheit zur näheren Bearbeitung des biologischen Materials gefunden. Der speziell chemische Teil wird hiermit mit seiner Genehmigung mitgeteilt. Er hat mir auch freundlichst mündlich von seinen Beobachtungen Mitteilung gemacht. Ferner wird hier Gebrauch gemacht von einer von ihm ausgearbeiteten vorläufigen Planktontabelle, die schon früher Herrn Prof. R. Witting[1]) für eine Studie über Wasserumsatz und Wasserverunreinigung im Helsingforser Hafenwassergebiet zur Verfügung gestellt worden ist. In derselben Arbeit von Witting findet sich auch ein Teil meines Beobachtungsmaterials.

Grösse und Form des Beckens geht aus der Kartenskizze Fig. 1 hervor. Es steht durch eine ca. 75 m breite Enge mit einem äusseren, von Inseln ziemlich umschlossenen Hafengebiet von ca. 15 km² in Verbindung. Die Totalfläche der Tölöwiek selbst beträgt ca. 0,5 km², die Tiefe 2 bis $2^1/_2$ m, das Wasservolumen etwa 800.000 m³. Durch einen Eisenbahndamm ist sie in zwei Teile geteilt. Die Kreise an den Ufern auf der Kartenskizze geben die Mündungsstellen der Abwasserleitungen an, die Punkte I und II die Probenentnahme- und Beobachtungs-Stellen. Folgende Bestimmungen wurden ausgeführt: Temperatur, Chlor, Sauerstoffgehalt, pH; am Punkte I ausserdem Totalkohlen-

[1]) R. Witting: Fjärdarna kring Helsingfors med hänsyn till vattenomsättningen och föroreningen. Havsforskningsinstitutets skrift N:o 11. 1922.

säure ΣCO_2 und Titrationsalkalinität, ferner zur allgemeinen Orientierung über die Beschaffenheit des Wassers einzelne Bestimmungen von Ammoniak, Nitrat, Nitrit, sowie des Permanganatverbrauches.

Ein erster Besuch fand am 16. Juni 1921 statt; zum ersten Mal von 4 bis 5 Uhr morgens, zum zweiten Mal am selben Nachmittag von 19 bis 20. Lage und Beschaffenheit des Wassers geht aus der Tabelle 1 hervor. Temperatur und Salzgehalt haben sich zwischen den beiden Zeitpunkten nur unbedeutend verändert. Der Sauerstoff weist im äusseren Punkt an der Oberfläche, im inneren in der ganzen Schicht überaus hohe Beträge auf,

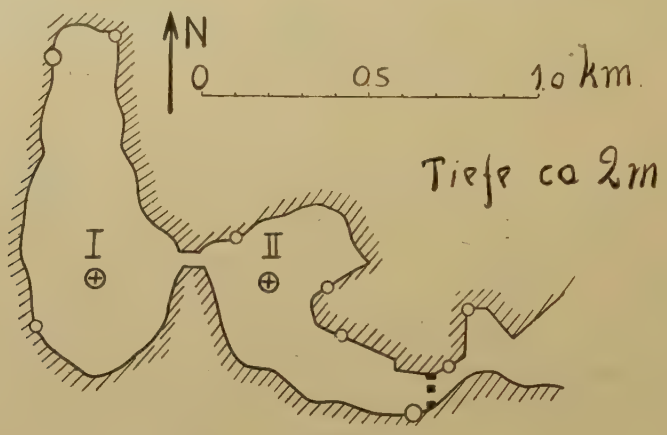

Fig. 1.

die im Laufe des Tages zufolge der intensiven Assimilationstätigkeit noch stark anwachsen. In Punkt II, in der Nähe des Bodens, herrscht jedoch Untersättigung und es findet rapide Sauerstoffabnahme statt, die auf beginnende Fäulnis deutet. Sonst besteht im ganzen eine intensive Selbstreinigung. Das Vorhandensein dicht an einander grenzender Schichten von 160 und 18 % Sauerstoff zeigt jedoch eine gewisse Labilität des Zustandes an.

Tabelle 1.

Lage am 16. Juni.

	Punkt I				Punkt II			
	0 Meter		2½ Meter		0 Meter		2 Meter	
Stunde	4	19	4	19	5	19½	5	19½
t°	17,9	17,9	17,7	17,9	15,4	15,4	12,7	14,2
S ‰	4,61	4,67	4,69	4,70	4,94	4,99	5,23	5,08
O_2 %	157	168	120	169	141	161	74,6	17,8
$KMnO_4$ Verbrauch mg/l	..	67	..	45	..	43	..	23
NH_4 mg/l N	..	0,29	..	0,50	..	0,05	..	0,11
NO_3 mg/l N	..	0,22	..	0,28	..	0,0	..	0,05

Wenngleich sich auch die tageszeitlichen Variationen beim ersten Besuch deutlich erkennen liessen, obwohl die Grenzen zwischen Tageshelle und Nachtdunkel zu dieser Jahreszeit und in so hoher Breite nicht sehr scharf sind, wurde die Fortsetzung der Untersuchungen auf Ende August verlegt, wenn stärkere Belichtungsunterschiede herrschen, und dann mehrere Male am Tage Probenentnahmen und Beobachtungen ausgeführt. Diese wurden am 23. August abends begonnen und am 25. August morgens beendet.

Das Wetter war während der ganzen Untersuchungszeit klar, der Himmel unbewölkt, mit schwachen Winden von wechselnder Richtung. Die Wasserstandsschwankungen waren unbedeutend, geringer als 7 cm. Die Wasserumlagerung war also gering und gleichmässig, was auch aus den Salzgehaltszahlen hervorgeht.

Tabelle 2.

	Punkt I. Oberfläche					Punkt I. 2 m Tiefe				
Tag	23	24			25	23	24			25
Stunde	19^{10}	5^{15}	11^{35}	19^{30}	5^{15}	19^{10}		11^{35}	19^{30}	5^{15}
t°	20,0	18,2	19,2	19,1	18,2	18,0		18,2	18,2	17,8
S ‰	4,90	4,90	4,92	4,92	4,92	5,03		5,01	4,96	4,98
Alk. m äqu/l	1,48	1,52	1,53	1,52	1,51	..		..	..	..
CO_2 cm³/l	27,8	30,5	35,1	36,3	37,1	..		..	..	..
O_2 cm³/l	6,40	3,71	2,68	2,09	0,70	1,58		0,13	0,14	0,00
O_2 %	103	58	42	33	11	24,5		2,0	2,2	0,00
p_H	8,81	8,51	8,30	..	7,68	8,39		8,07	7,68	7,74
O_2-Abnahme		2,7	1.03	0,59	1,39	Gesamtabnahme O_2: 5,7 cm³/l				
CO_2-Zunahme		2,7	4,6	1,2	0,8	— zunahme CO_2: 9,3 cm³/l				
CO_2/O_2		1,0	3,5	2,0	0,6	Gesamt CO_2/Gesamt O_2 1,6				

	Punkt II. Oberfläche					Punkt II. 2 m				
Tag	23	24			25	23	24			25
Stunde	19	5	11	19	5	19	5	11	19	5
t°	18,4	17,3	18,1	18,0	17,3	15,8	16,0	16,8	16,3	17,0
S ‰	5,17	5,12	5,07	5,06	5,08	5,21	5,21	5,21	5,16	5,08
O_2 cm³/l	7,30	6,02	6,37	7,34	4,88	5,59	4,11	4,15	4,11	3,84
O_2 %	114	92	99	114	75	83	61	63	62	58
p_H	8,54	8,38	8,37	8,45	7,97	8,38	7,70	7,74	7,57	7,68

Die ausgeführten Beobachtungen und chemischen Untersuchungen sind in Tabelle 2 zusammengestellt. Eine Analyse von Stickstoffverbindungen in Punkt I, 0 m, ergab NH_4 = 1,1mg/l, NO'_3 = 0,0 mg/l, NO'_2 = 0,005 mg/l Stickstoff.

Tabelle 3 enthält vorläufige approximative Mengenangaben (Anzahl Individuen in einem Liter Wasser) der häufigsten Pflanzen- und Tierplanktonarten. In der Tabelle

bedeutet O. A. *Oscillatoria Agardhii.* (Die Angabe bezieht sich hier auf die Anzahl Fäden in einem Liter Wasser.) A. s. = *Anabaena spiroides.* Eine häufige, in der Tabelle nicht aufgenommene Algenart war noch *Euglena viridis.* Die häufigsten Tierorganismen waren *Brachionus pala* = Br. p. und *Triarthra longiseta* = Tr. l.

Tabelle 3.

Tag	23	24			25
Stunde	19^{10}	5^{15}	11^{35}	19^{30}	5^{15}
O. A. 0 m	813.000	556.000	153.000	84.000	58.800
O. A. 2 m	999.000	663.000	430.000	652.000	243.600
A. s. 0 m	2.000	17.000	17.000	6.000	21.000
A. s. 2 m	8.000	17.000	4.000	15.000	4.000
Br. p. 0 m	8.000	8.000	10.000	18.900	94.500
Br. p. 2 m	2.000	4.000	4.000	0	4.000
Tr. l. 0 m	6.000	2.000	..	4.000	4.000
Tr. l. 2 m	..	..	2.000	0	2.000

Beim ersten Besuch am 23. Aug. um 7 Uhr abends herrschten ungefähr folgende Verhältnisse. Das Wasser war ziemlich stark grün gefärbt. Im inneren Teil Punkt I Sauerstoffsättigung in der Oberfläche, in 2 m Tiefe starke Untersättigung, 25 % O_2. Die Oscillatoriafäden waren hier stark eingeschrumpft und kleiner als an der Oberfläche. Der äussere Teil (Punkt II) war in der Oberfläche übersättigt, in 2 m etwas untersättigt. Die überaus hohe Übersättigung, bis 160 %, die im Juni im grössten Teil des Wassergebietes gefunden wurde, war also jetzt nicht mehr vorhanden. Typisch ist die stark alkalische Reaktion, die auf eine stattgefundene intensive Assimilationstätigkeit deutet.

Der Besuch am nächsten Morgen wies überraschende Änderungen im Zustande des Stoffumsatzes auf, die sich im Laufe des Tages noch fortsetzten. Das Wasser war graugrün gefärbt und ging allmählich ganz in grau über. Der Sauerstoff war im Punkt I, 0 m, über Nacht fast auf die Hälfte geschwunden, in der ganzen Beobachtungszeit von 34 Stunden sank er auf $^1/_{10}$. In 2 m Tiefe war der schon anfangs geringe Gehalt in dieser Zeit gänzlich verschwunden. Im äusseren Punkt mit dem von Beginn reineren Wasser waren die Verhältnisse stabiler. Jedoch auch hier setzte der Sauerstoffsturz, wenngleich einen Tag später, ein. Der Sauerstoffveränderung entsprechend veränderte sich pH in saurer Richtung hin, was besonders deutlich in Punkt I, 0 m, hervortritt. Der Gehalt an Totalkohlensäure stieg während der ganzen Beobachtungszeit um 9,3 cm^3/l. Die entsprechende Sauerstoffabnahme war nur 5,7 cm^3/l. Die Ursache hierzu wird weiter unten besprochen. Die Veränderungen von einem Zeitpunkt zum folgenden sind in dem unteren Teile der Tabelle 2 (Punkt I) aufgenommen. Graphisch sind sie in Fig. 2 dargestellt.

Die beobachtete plötzliche Zustandsveränderung weist auf eine Vergiftung der Algenvegetation hin, die fast das gänzliche Aufhören der Assimilationstätigkeit zur Folge

hatte[1]). Allein der Stoffumsatz des tierischen Lebens blieb bestehen. Dieser Umstand gibt uns die Möglichkeit, uns eine Vorstellung von der Stoffwechselintensität des tierischen Lebens unabhängig von dem pflanzlichen Leben zu machen. Die Stoffwechselintensität war am stärksten an Punkt I in der Oberfläche. Als ihr Mass konnten die O_2-bzw. CO_2-Zahlen jedoch nur bedingt dienen, weil der Austausch mit der Atmosphäre als Nebenfaktor störend einwirkte. In der Tiefe war bei Beginn der Beobachtungen der Sauerstoff schon so herabgesetzt, dass aller Stoffwechsel ausser dem der Bakterien aufgehört hatte, d. h. vollständige Fäulnis eingetreten war.

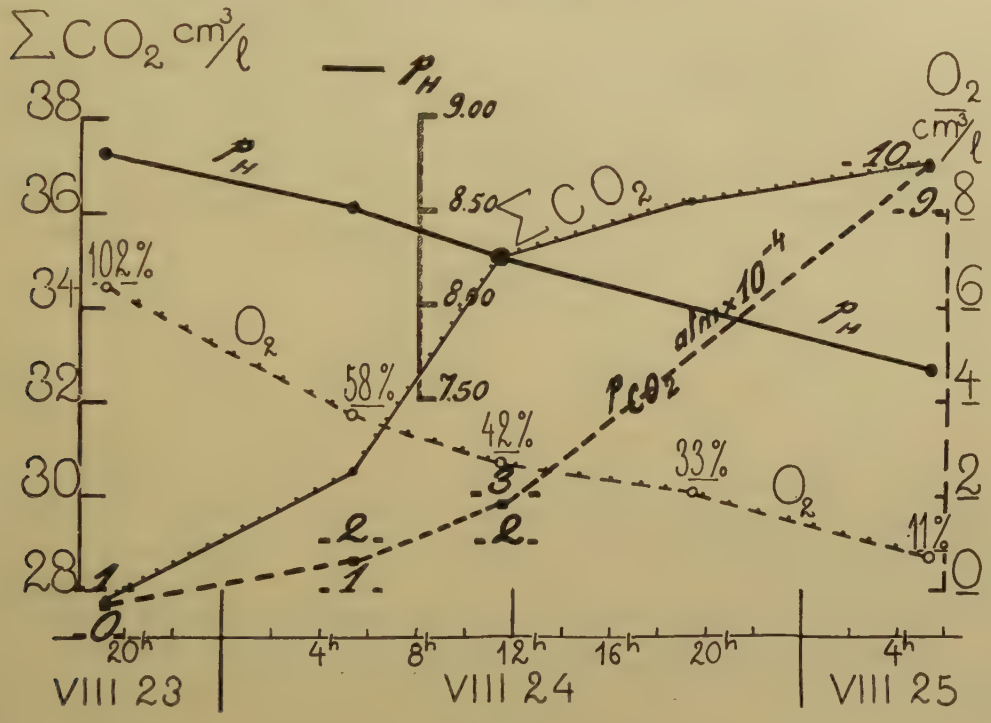

Fig. 2.

Am zuverlässigsten sind die Veränderungszahlen in der Oberfläche zu Beginn der Periode, mit gleich grosser Sauerstoffkonsumtion und CO_2-Produktion 2,7 cm³/l. Das Verhältnis CO_2/O_2, der sogen. respiratorische Koeffizient, hat den normalen Betrag 1,0. In der zweiten Untersuchungsperiode hat die O_2-Konsumtion scheinbar nachgelassen und die CO_2-Produktion hat zugenommen, und wir kommen zu einem abnorm hohen Koeffizienten $CO_2/O_2 = 3{,}5$. Weiterhin nimmt der Wert des Koeffizienten wieder ab. Letzteres Verhältnis kann zum Teil dem Einfluss der Atmosphäre zugeschrieben werden. In Betreff des Sauerstoffs herrschte zu Beginn Gleichgewicht mit der Atmosphäre, später Untersättigung, was Nachlieferung von Sauerstoff aus der Atmosphäre zufolge hatte, wodurch die Zahlen

[1]) Der hierdurch entstehende Sauerstoffmangel ist wohl die Ursache zu dem in der Tölöwiek mehrmals beobachteten Massensterben von Fischen.

für die Konsumtion natürlich zu klein werden. Was wieder die Kohlensäure betrifft, war auch hier anfangs kein Einfluss von Seiten der Atmosphäre vorhanden. Das Wasser war zufolge seiner alkalischen Reaktion zwar CO_2-absorbierend, aber der im Verhältnis zum Wasser überaus geringe Gehalt der Atmosphäre von ca. 0,3 cm³ pro l Luft kann keinen merkbaren Einfluss ausgeübt haben. Die CO_2-Produktionszahlen in den beiden ersten Perioden geben also die Oxydationsintensität richtig an. Es musste sich aber bei der Fortdauer der CO_2-Produktion, die sich auch in den pH-Zahlen zu erkennen gibt, sehr bald Gleichgewicht mit der Atmosphäre einstellen und darauf CO_2-Abgabe an die Atmosphäre stattfinden. Offenbar ist die scheinbare Verminderung der CO_2-Produktion und des respiratorischen Quotienten diesem Umstande zuzuschreiben. Um dies zu kontrollieren, wurde eine Berechnung der CO_2-Tension des Wassers aus den pH-Zahlen und der Alkalinität auf dem von Buch[1]) angegebenen Wege, mit Hilfe der von K. Buch, H. W. Harvey, H. Wattenberg[2]) experimentell festgestellten Dissoziationskonstanten der Kohlensäure ausgeführt und dabei folgende approximativen Zahlen gefunden:

Werte der CO_2-Tension in der Oberfläche von Punkt I in zehntausendstel Atmosphären.

Tag	23	24		25
Stunde	19^{10}	5^{15}	11^{35}	5^{15}
CO_2-Tension	0,7	1,5	2,8	10

Der Verlauf der Tension pCO_2 ist auch in der Kurvenskizze dargestellt.

Da die CO_2-Tension der Atmosphäre ca. 3 zehntausendstel Atmosphären beträgt, bestätigen sich also die gegebenen Angaben. Gleichgewicht mit der Atmosphäre war am Vormittag des 24. eingetreten. Später fand CO_2-Abgabe statt und, wie erwähnt, O_2-Aufnahme, welcher Umstand die Stoffwechseldaten verwischt.

Von Interesse ist es nun, diese rein chemisch gewonnenen Daten mit den Planktonbeobachtungen von Herrn Välikangas zu vergleichen. Wenngleich die Zahlen nach den Angaben von Herrn Välikangas nicht endgültig sind, kann aus ihnen doch deutlich erstens das erwähnte Verschwinden der Algenvegetation aus der Oberfläche herausgelesen werden. Die Anzahl der *Oscillatoria*-Fäden verminderte sich in der Oberfläche von über achthunderttausend bis unter sechzigtausend, desgleichen in 2 Meter Tiefe von etwa 1 Million zu ca. $^1/_4$ Million. Die Abnahme in 2 Meter Tiefe war aber viel geringer. Das Mengenverhältnis in 0 Meter und 2 Meter verschob sich zu gunsten der Tiefe, anfangs 1 : 1,25, zuletzt 1 : 24, d. h. die Algen starben ab und sanken in die Tiefe. Die Algen in 2 Meter waren ausserdem, wie erwähnt, zusammengeschrumpft und kleiner, also offenbar gerissen. Die *Anabaena* zeigten schwankende Verhältnisse, waren aber im Verhältnis zu *Oscillatoria* von untergeordneter Bedeutung für das Gesamtresultat.

Im Betreff der Tierorganismen ist bemerkenswert das starke Anwachsen der Menge

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[1]) K. Buch: Die Kohlensäurefaktoren des Meerwassers I und II, Rapports et Procès-Verbaux du Conseil International pour l'Exploration de la Mer. Vol. LXVII, 1930.

[2]) Zur Zeit noch nicht veröffentlichte im Auftrage des Conseil International pour l'Exploration de la Mer ausgeführte Untersuchung.

von *Brachionus* in der Oberfläche, besonders zu den beiden letzten Zeitpunkten. Von Interesse ist hier eine Beobachtung von Herrn Välikangas, nämlich, dass sich sämtliche Individuen dicht an die Oberfläche drängten, offenbar um Luft zu schöpfen, die ihnen im Wasser nicht mehr genügend zu Gebote stand. Dieser Umstand trägt auch zur erwähnten scheinbaren Abnahme der Sauerstoffkonsumtion bei. Die Organismen nahmen den Sauerstoff direkt aus der Luft oder aus den allerobersten Wasserschichten, wo er sofort verbraucht und so der der Nachweisbarkeit durch die Analyse entzogen wurde. Das dabei produzierte Kohlendioxyd konnte dagegen anfangs nicht in die Atmosphäre gelangen, weil, wie erwähnt, das Wasser stark CO_2-absorbierend war.

Wir finden also, dass die chemischen und biologischen Befunde sich in ausgezeichneter Weise ergänzen. In dem hier beschriebenen Spezialfalle, in dem das pflanzliche Leben plötzlich grösstenteils aus dem Stoffwechsel ausschied, konnten die chemischen Befunde ein gutes Bild der Stoffwechselintensität geben. Wenn aber, wie gewöhnlich in den obersten Wasserschichten, ungefähr Gleichgewicht zwischen O_2-Konsumtion und Produktion, bzw. zwischen CO_2-Produktion und -Konsumtion herrscht, so wird die Deutung der chemischen Befunde mit grossen Schwierigkeiten verbunden sein, da der Lagerbestand sich, unabhängig davon, ob grosser oder kleiner Umsatz vorhanden ist, unverändert halten kann. Es wird so das Zusammenwirken der chemischen mit quantitativen Planktonuntersuchungen zur unbedingten Notwendigkeit, und zu einer wichtigen Forderung der Stoffwechselforschung im Meere, die zur Zeit erst in ihrem Beginn ist.

Summary.

The investigation described below was undertaken with a view to determining the daily variations, which the chemical and biological factors of a water are subjected to under favorable conditions of intense metabolism. The object of investigation was an inner part of the harbour of Helsingfors, the Tölö Bay, the water of which was strongly polluted by sewage and which had a rich population of phyto- and zoo-plankton. The work was carried out by Mr. I. Välikangas, Ph. D. who investigated the biological factors and by the author who investigated the chemical factors. As Mr. Välikangas has not yet had the opportunity to treat his material in detail only the chemical result are given here. Mr. Välikangas has, however, kindly informed me of his observations. Also I have used his preliminary table of plankton organisms which was put at Professor R. Witting's disposal and previously published in connection with a study of the pollution of the harbour waters of Helsingfors.

The character of the basin is given on page 59. Samples were taken at stations I and II, see diagram Fig. 1. A preliminary series of observations was made on June 16th, when samples were taken at 4—5 and 19—20 o'clock. The hydrographic situation is shown in Table 1. The most striking feature is the very high content of oxygen in some samples, highest in those taken in the evening. An intense self purification is going on. At station II adjoining layers have 160 % and 18 % of oxygen, which points to a high lability. The second series was done in August when the daily variations in illumination were greater. The results can be seen from Table 2. Some nitrogen-determinations are

quoted on page 61. Table 3 gives approximate quantities of some species of plankton. For meaning of abbreviations see page 62. Fig. 2 shows graphically the development at station I, 0 m.

The results show that at the time the observations began a striking change in the normal development was taking place. In the evening of August 23rd at station I the water was saturated with oxygen at the surface and unsaturated at 2 metres depth (O_2 = 25 %), its colour being green, whereas the next morning only half of the oxygen was left at the surface and none at 2 metres and the colour had changed to greyish. In stead of increasing during the day it further decreased. At the same time the CO_2-content (station I, 0 m) increased and the strong alkaline reaction (see the pH-values) decreased. A sudden poisoning of the phytoplankton had occurred, which had stopped assimilation almost completely. This incident rendered it possible to get an idea of the animal metabolism as illustrated by the O_2-, CO_2- and p_H-values. The so-called "respiratory quotient", the ratio between the CO_2-production and the O_2-consumption, was normal during the first period (= 1.0, see Table 2, station I, 0 m), but rose afterwards to an abnormally high value. The reason was found partly in atmospheric influence, partly in the behaviour of the plankton organisms. Because of the lack of oxygen the zooplankton had gathered at the surface and now absorbed oxygen either directly from the atmosphere or from the very top layer, which oxygen in both cases escaped analysis. The carbon dioxide, at the same time produced, however, to begin with remained in the water, this being very alkaline. With rising CO_2-content also the CO_2-tension increased. This was calculated by a method given by Buch, using not yet published values of the dissociation constants of carbonic acid, jointly determined by Buch, H. W. Harvey and H. Wattenberg. See page 64.

When the CO_2-tension had increased above that of the atmosphere, also CO_2 escaped and the "respiratory quotient" decreased again. Because of these side influences, however, only the CO_2- and O_2-variations of the first period can be regarded as giving a correct picture of the metabolic intensity. On the whole the biological and chemical parts of the investigation agree very well. A successful study of metabolism in the sea cannot dispense with one or the other.

V. Beziehungen zwischen Kalkgehalt des Meerwassers und Plankton.

Von

H. Wattenberg.

Eine grosse Reihe von Untersuchungen haben in den letzten Jahren die Beziehungen zwischen den zum Aufbau des pflanzlichen Protoplasmas nötigen Nährstoffen, besonders Phosphor- und Stickstoffverbindungen, und der Produktion des Phytoplanktons zum Gegenstand gehabt. Dabei hat sich gezeigt, dass eine gewisse Konzentration an diesen Stoffen zwar notwendig, aber nicht allein massgebend ist. Auf der Suche nach anderen Faktoren wurde vor allem der Einfluss der Beleuchtung (Atkins) und die Wirkung von stimulierende Substanzen (Eisenverbindungen, Gran) in Betracht gezogen.

Für sehr verbreitete Gruppen von Planktonorganismen, nämlich die, welche Schalen oder Skelette aus Kieselsäure oder Kalziumkarbonat bilden, könnte auch der Gehalt des Meerwassers an diesen beiden Stoffen ein produktionsbestimmender Faktor sein. Tatsächlich hat Atkins bei sehr starker Diatomeenentwicklung fast vollständigen Verbrauch der gelösten Kieselsäure beobachtet. Im allgemeinen wird es jedoch nicht so weit kommen, denn die in viel geringerer Menge als das Silikat vorkommenden Nitrate und Phosphate sinken zuerst unter die Minimalkonzentration und begrenzen die weitere Vermehrung des Planktons, ehe eine Verarmung an Silikat eintreten kann. Beim Kalziumkarbonat liegt eine solche Gefahr der Verarmung noch viel weniger vor, denn der Gehalt an Kalziumkarbonat ist noch einige Hundert mal grösser als der an Silikat. Es konnte zwar eine erhebliche Verminderung der $CaCO_3$-Konzentration in den von Kalkorganismen bevölkerten warmen Meeren festgestellt werden[1]), aber selbst wenn, wie stellenweise beobachtet, 1800 mg/m³ dem Wasser entzogen sind, bedeutet das erst ein Sinken der $CaCO_3$-Konzentration um weniger als 2 %. Das ist natürlich für das Plankton gänzlich ohne Bedeutung.

An Kalziumkarbonat kann also — der Menge nach — nie ein Mangel eintreten. Wohl aber kann der grössere oder kleinere Kohlensäuregehalt des Wassers die Verwertbarkeit des gelösten Kalks für die Organismen vermindern bzw. vermehren. Die Löslichkeit des Kalks im Meerwasser ist nämlich stark vom Kohlensäuregehalt abhängig. Durch Laboratoriumsversuche wurde diese Abhängigkeit zahlenmässig er-

[1]) Annalen d. Hydr. u. s. w. 1931. (Im Druck).

mittelt[1]) und durch Vergleich mit den wirklich im Meere gefundenen Mengen festgestellt, wo das Wasser an Kalk untersättigt oder übersättigt ist. Dabei ergab sich, dass das Oberflächenwasser überall mehr, zum Teil sehr viel mehr Kalk enthält, als der Löslichkeit bei dem an der betreffenden Stelle herrschenden Kohlensäuregehalt entspricht. Trotzdem scheidet sich normalerweise der zuviel vorhandene Kalk nicht aus, wenn Kristallisations-Keime fehlen. Aber es ist doch wohl sicher, dass die lebenden Zellen, die Kalk zum Aufbau von Skeletten brauchen, aus einem solchen übersättigtem Wasser den Kalk mit besonderer Leichtigkeit ablagern können, und zwar umso leichter, je stärker das Wasser an Kalk übersättigt ist. Da der Grad der Uebersättigung von der gelösten Kohlensäure abhängt, und die gelöste Kohlensäure-Menge an der Oberfläche mit steigender Temperatur abnimmt, sind die warmen Meeresgebiete besonders stark übersättigt. Sie enthalten bis zu 3 mal mehr Kalk, als der Löslichkeit entspricht. Andererseits ist es bekannt, dass das Kalkplankton vorwiegend in diesen Gebieten zu Hause sind. Der erwähnte Zusammenhang gewinnt damit sehr an Wahrscheinlichkeit. Für eine bestimmte Gruppe pflanzlicher Kalkorganismen seien auch einige Zahlen angeführt, die den Beobachtungen von E. Hentschel[2]) während der Expedition entnommen wurden. Sie werden verglichen mit dem in den gleichen Zonen herrschenden Sättigungszustand des Wassers an Kalk:

Geographische Breite	Das Wassers enthält n mal mehr Kalk als Sättigung entspricht. n =	Gehalt des Wassers an Coccolithophoriden in Prozenten des Gesamtplanktons
ca. 55° S.	1,2	5 %
- 30° S.	3,0	45 %
- 0°	3,8	40 %

Man kann kaum erwarten, dass bei solchen einmaligen Beobachtungen, die noch dazu zu verschiedenen Jahreszeiten angestellt wurden, der Zusammenhang viel besser herauskommt. Dagegen könnte man die Frage vielleicht durch Kulturversuche mit derartigen Organismen näher prüfen, nachdem in den letzten Jahren die Technik solcher Versuche sehr gefördert worden ist (Schreiber, Gran u. Mitarbeiter, Harvey). Da auch das System $CaCO_3$-CO_2 jetzt gut bekannt ist, hat man die Möglichkeit, den Sättigungsgrad des Wassers an $CaCO_3$ variieren, aber alle übrigen Faktoren (Temperatur, Kohlensäuregehalt, pH) konstant zu halten.

Mehr noch als bei pflanzlichen Kalkorganismen müsste die Abhängigkeit von der Kalksättigung bei den tierischen zum Ausdruck kommen. Die Pflanzen können ja immer durch Assimilieren Kohlensäure dem Wasser in ihrer nächsten Umgebung entziehen und dadurch eine lokale Uebersättigung an Kalk hervorrufen, während die Heterotropen durch ihre Kohlensäureproduktion auf besonders starke Uebersättigung angewiesen sind. Daran mag es auch zum Teil liegen, dass die Korallen so sehr an warmes Wassers gebunden sind, das eben immer besonders stark kalkübersättigt ist. Wenn sie aber in käl-

[1]) Erscheint in der Z. f. anorgan. u. allgem. Chemie.
[2]) Wiss. Erg. d. Deutschen Atl. Exp. Bd. X (1931).

terem, weniger übersättigtem Wasser vorkommen, so äussert sich die Schwierigkeit, den Kalk dem Wasser zu entziehen, in zarten und zerbrechlichen Kalkgerüsten.

Zum Schluss mag noch erwähnt werden, wie diese starke Übersättigung des Oberflächenwassers zustande kommt und aufrecht erhalten bleibt: In den grossen Tiefen des Oceans ist das Wasser wegen des höheren Kohlensäuregehalts und des starken Wasser-Druckes an Kalk ungesättigt. Beim Aufsteigen lässt der Druck nach, das Wasser wird wärmer und gibt an der Oberfläche Kohlensäure an die Atmosphäre ab. Die Löslichkeit für $CaCO_3$ sinkt daher, aber da der $CaCO_3$-Gehalt derselbe bleibt, entsteht eine entsprechende Übersättigung.

VI. On the Rate of Photosynthesis by Diatoms.

By

H. W. Harvey.

(Marine Biological Association, Plymouth).

An account was read of a preliminary investigation concerning the effect of various additions of nutrient salts — phosphates, arsenates, nitrates and ammonium salts — upon the rate of photosynthesis by diatoms.

Their rate of growth was measured by noting the oxygen evolved by cultures at intervals during their growth.

The results indicate that the concentration of these nutrient salts affect the rate of photosynthesis as well as limiting the total growth. In the case of phosphate the experiments suggest a low optimum concentration; further confirmation is necessary.